PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2020, **54** (2), p. 132–137

Biology

STUDY OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF SAXICOLOUS LICHEN *XANTHOPARMELIA STENOPHYLLA*

A. G. SIMONYAN [*], R. R. SARGSYAN [**], H. H. PANOSYAN [***], A. H. TRCHOUNIAN [****]

Chair of Biochemistry, Microbiology and Biotechnology, YSU, Armenia

Antibacterial and antioxidant activities of different extracts of saxicolous lichen *Xanthoparmelia stenophylla* sampled from Norashen, Gegharkunik Province of Armenia were studied. Methanol, ethanol and acetone extracts of lichen thalli were demonstrated to have activity against only tested gram-positive bacteria. Methanol extract of the lichen showed the highest amount of DPPH radical scavenging activity (~68%). Our studies did not reveal any significant antibacterial and antioxidant activities of aqueous extract.

https://doi.org/10.46991/PYSU:B/2020.54.2.132

***Keywords*:** *Xanthoparmelia stenophylla*, saxicolous lichens, antibacterial and antioxidant activities.

Introduction. Since ancient times, lichens have been used for various remedies in folk medicine [1]. Screening of lichens has revealed the frequent occurrence of metabolites with antifungal, antibacterial, antiviral, antitumor, anticytotoxic, analgesic, and antipyretic properties [2–4]. Nowadays there is a growing interest among scientists towards the possible biological activity and biochemical properties of lichen secondary metabolites [1, 5, 6].

Lichens have long been undervalued by biotechnologists and overlooked by pharmaceutical industry because of their slow-growing nature and difficulties in their cultivation in laboratory conditions [6, 7]. Difficulties in obtaining lichens metabolites in quantities and purities sufficient for structural elucidation and pharmacological testing were the second main reason for limiting their studies [6, 8]. That is why relatively few lichen substances have been observed in detail from the therapeutic point of view. There are some reports describing antibacterial activities expressed by corticolous lichens worldwide [1]. However, research reports on saxicolous lichen flora are very limited.

The abundance of biodiversity of Armenia includes also a wide variety of lichen species, which are unique to region or even endemic [9]. There is a lack of studies regarding to this mega-diversity hotspot of the world. Studies of the biotechnological potential of saxicolous lichens have also not been conducted yet. Based on the foregoing, the main purpose of this study was to investigate the

[*] E-mail: anna.simonyanan3@ysumail.am
[**] E-mail: razmik.sargsyan2@ysumail.am
[***] E-mail: hpanosyan@ysu.am
[****] E-mail: trchounian@ysu.am

antibacterial and antioxidant activities of the saxicolous lichen *Xanthoparmelia stenophylla*, common in Armenia.

Materials and Methods.

Collection of lichen samples. Samples of *X. stenophylla* crustose saxicolous lichen were collected from Gegharkunik Province of Armenia, near Norashen village (40°52'06.9"N 45°27'09.8"E, altitude 1928 *m* AMSL) (Fig. 1). Sampling was held from September to October 2018. The sampling site was chosen considering the high humidity and high altitude of area, which promoted rapid growth of lichens. The herbarium of lichen thalli is currently maintained at the Department of Biochemistry, Microbiology and Biotechnologyof YSU.

Fig. 1. *X. stenophylla* lichen on the rock at the sampling site.

Preparation of Lichen Extracts. Lichen thalli (8 *g*) were dried and milled using an automated grinder. To obtain extracts, the powder-like substance was soaked with methanol, ethanol and acetone at 1:5 sample-to-solvent ratio (w/v). After 24 *h* extraction on a magnetic stirrer, the extracts were centrifuged (15 *min*, 12000 *rpm*) and dried by a vacuum evaporator (BOV-50 V vacuum drying oven, Biobase Meihua Trading, China) at 37℃ temperature. In the case of aqueous extract, the grinded lichens were dissolved in distilled boiled water, then left for extraction for 24 *h*. The resulting water tinctures were filtered through 0.22 sterile millipore filters and dried under aseptic conditions. All dry extracts were weighted and stored at –18℃. The stock solutions were prepared by dissolving crude dried masses in pure dimethyl sulfoxide (DMSO) (“Sigma-Aldrich”) and kept under –4℃ conditions. For experiments, the final concentration of DMSO in extracts was 5%.

Antibacterial Activity. Gram-positive (*Bacillus subtilis* WT-A and *Staphylococcus aureus* MDC 5233) and gram-negative (*Escherichia coli* VKPM-M17 and *Salmonella typhimurium* MDC 1754) bacterial strains from the collection of microbes at the Department of Biochemistry, Microbiology and Biotechnology, YSU, were used as test organisms to study the antibacterial activity of lichen extracts. The antibacterial activity was estimated using disc diffusion assay by incubation of bacterial strains on solidified Mueller-Hinton broth (MHB) [10]. Sterilized Whatman filter papers soaked with 5 μL of diluted in DMSO extracts were placed on plates for each microbial strain and incubated at 37℃ for 24 *h*. The experiments were performed by three replicates, and the diameter of the inhibition zones (IZ) around the disks was taken as a unit of measure of the antibacterial activity. Filter papers soaked in 5% sterilized DMSO served as a control.

Radical Scavenging Activity. The free radical scavenging activity of lichen extracts was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) [11]. The test sample solution, containing 1 $mg \cdot mL^{-1}$ extract and 0.1 *mM* DPPH, after vigorously shaking, was stored at room temperature for 30 *min* away from light, and the absorbance was measured by a spectrophotometer at a wavelength of 517 *nm*. Ascorbic acid was used as a positive control. For the negative control, extract was replaced with methanol. Radical scavenging activity (%) was considered equal to $[(A_0 - A_1)/A_0] \cdot 100$, where A_0 is the absorbance of the negative control and A_1 is the absorbance of the reaction mixture or standards.

Results and Discussion. The antibacterial activities of various extracts of saxicolous lichen *X. stenophylla* are presented in Tab. The activity was evaluated by the presence/absence of IZ and its diameter (Ø). The aqueous extracts did not exhibit any significant activity in disc diffusion assays. Methanol, ethanol and acetone extracts showed activity against gram-positive bacteria i.e. *B. subtilis* and *S. aureus*, whereas they did not show any activity against gram-negative bacteria. The highest antibacterial activity was observed in methanol extracts against *B. subtilis* (17 *mm*, Ø), *S. aureus* (20 *mm*, Ø), while for ethanol and acetone extracts it was estimated at 7 and 12 *mm* for *B. subtilis*, 20 and 15 *mm* for *S. aureus*, respectively.

Antibacterial activity of X. stenophylla lichen extracts

Crude extracts	Test microbes, IZ (Ø, *mm*)			
	B. subtilis	*S. aureus*	*E. coli*	*S. typhimurium*
Methanol	17 ± 0.5	20 ± 0.5	–	–
Ethanol	7 ± 0.5	20 ± 0.5	–	–
Acetone	12 ± 0.5	15 ± 0.5	–	–
Water	–	–	–	–
NC	–	–	–	–

(–): no zone of inhibition; NC: negative control (DMSO)

The scavenging activity of DPPH radicals of the tested lichen extracts is shown in Fig. 2. Acetone, ethanol and methanol extracts exhibited a relatively high scavenging activity, at that methanol extract of the tested lichen showed the highest amount of scavenging activity (~ 68%). No activity was found for aqueous extracts.

The species of genus *Xanthoparmelia* like *X. camtschadalis*, *X. delisei*, *X. loxodes*, *X. pokornyi*, *X. stenophylla*, *X. tinctina* and *X. verruculifera* are widespread in Turkey, Iran, Armenia and in Caucasus in general [12]. Some species of this group such as *X. conspersa* (Ehrh. ex Ach.) Hale have been used in folk medicine to treat snake bites, cuts, inflammatory gingivitis, and sore throat [13]. In this study, we targeted to evaluate the antibacterial and antioxidant activities of *X. stenophylla*.

The lack of any significant biological activity of the aqueous extract of *X. stenophylla* in our studies corresponds to data described earlier by some authors [14]. However, there are also contradictory data showing that aqueous extract of some *Umbilicaria, Xanthoria* and *Xanthoparmelia* species have antibacterial activity against *E. coli, B. subtilis* and *S. aureus* [15]. Probably, in our case, compounds with antibacterial activity contained in lichen thallus were not soluble or were poorly soluble in water [16].

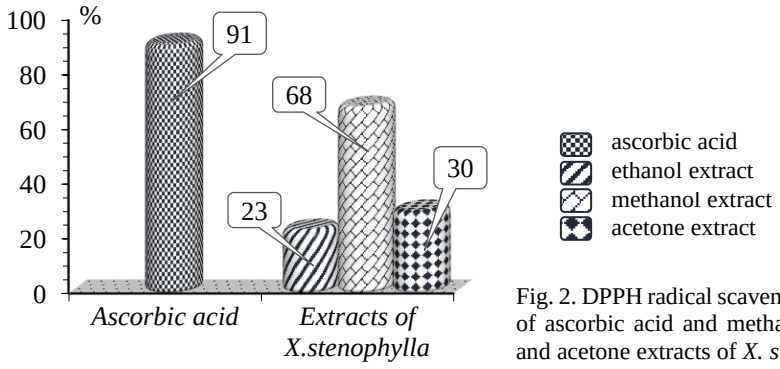

Fig. 2. DPPH radical scavenging activity of ascorbic acid and methanol, ethanol and acetone extracts of *X. stenophylla*.

In contrast to aqueous extracts, other extracts displayed a relatively high biological activity. The strength of antibacterial activity varied among different extracts, where the highest activity was observed in methanol extracts. Presumably, methanol was the most efficient solvent for the extraction of phenolic compounds which were responsible for antibacterial and antioxidant activities [17]. Antibacterial activity only against gram-positive bacteria can be explained by extracted compounds having effect on cell wall structures [18].

Recently, it was shown that acetone and chloroform extracts of *X. stenophylla* sampled from Turkey exhibited a maximum activity not only against gram-positive bacilli and streptococci, but also against gram-negative bacteria [19], which contradicts our results. This mismatch of results can possibly be explained by the geographical differences of sampling sites. Karaahmet et al. showed that acetone and ethanol extracts of *X. conspersa* expressed activity against only gram-positive bacteria, mainly *B. subtilis* [20]. In our case, the highest activity was recorded against *S. aureus*.

Among various species of lichens, various compounds have been observed that exhibit relatively high antioxidant activity. Previously, a correlation was found between phenolic, flavonoid compounds and antibacterial activity [21]. DPPH radical scavenging activities of acetone and chloroform extracts of *X. stenophylla* sampled from Turkey were ranged from 9.33 to 33.84% [19]. In our studies, the highest activity in the amount of 68% was observed for methanol extracts . In the case of *X. conspersa*, the highest DPPH radical scavenging activity in the amount of 64.9% was observed for its ethanol extract [20]. The contradiction with our results can be explained by species specificity, ecological determinants and dissimilar solvent.

Conclusion. In conclusion, it can be stated that methanol, ethanol and acetone extracts of *X. stenophylla* crustose saxicolous lichen collected from Gegharkunik Province, Armenia, displayed *in vitro* antibacterial activity mainly against to gram-positive bacteria and relatively high DPPH radical scavenging activity. In virtue of these results, lichens should be considered as organisms with high biotechnological potential, which was proved before by various authors, but has been reported for the first time for saxicolous lichens distributed on the territory of Armenia.

Received 20.12.2019
Reviewed 11.02.2020
Accepted 27.07.2020

REFERENCES

1. Ranković B., Kosanić M. Lichens as a Potential Source of Bioactive Secondary Metabolites. In: Ranković B. (ed.) *Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential* (2nd ed.). Springer, Cham (2019), 1–29. https://doi.org/10.1007/978-3-030-16814-8_1
2. Boustie J., Grube M. Lichens – A Promising Source of Bioactive Secondary Metabolites. *Plant Genetic Resources* **3** (2005), 273–87. https://doi.org/10.1079/PGR200572
3. Shukla V., Joshi G.P., Rawat M.S.M. Lichens as a Potential Natural Source of Bioactive Compounds: A Review. *Phytochem. Rev.* **9** (2010), 303–314. https://doi.org/10.1007/s11101-010-9189-6
4. Verma N., Behera B.C. Future Directions in the Study of Pharmaceutical Potential of Lichens. In: Ranković B. (ed.) *Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential* (2nd ed.). Springer, Cham (2019) 237–260. https://doi.org/10.1007/978-3-030-16814-8_9
5. Oksanen I. Ecological and Biotechnological Aspects of Lichens. *Appl. Microbiol. Biotechnol.* **73** (2006), 723–734. https://doi.org/10.1007/s00253-006-0611-3
6. Calcott M.J., Ackerley D.F., Knight A., Keyzers R.A., Owen J.G. Secondary Metabolism in the Lichen Symbiosis. *Chem. Soc. Rev.* **47** (2018), 1730–1760. https://doi.org/10.1039/C7CS00431A
7. Yamamoto Y., Kinoshita Y., et al. Screening of Biological Activities and Isolation of Biological-Active Compounds from Lichens. *Recent Res. Dev. Phytochem.* **2** (1998), 23–34.
8. Shrestha G., Clair L.L.S. Lichens: A Promising Source of Antibiotic and Anticancer Drugs. *Phytochem. Rev.* **12** (2013), 229–244. https://doi.org/10.1007/s11101-013-9283-7
9. Gasparyan A., Sipman H.J.M. New Lichen Records from Armenia. In: *Regional Annotated Mycobiotas New to the Mycotaxon Website*. *Mycotaxon* **123** (2013), 491–492. https://doi.org/10.5248/123.491
10. Rankovič B., Rankovic D., Maric D. Antioxidant and Antimicrobial Activity of Some Lichen Species. *Microbiology* **79** (2010), 809–815. https://doi.org/10.1134/S0026261710060135
11. Pisoschi A.M., Pop A., Negulescu G.P., Pisoschi A. Determination of Ascorbic Acid Content of Some Fruit Juices and Wine by Voltammetry Performed at Pt and Carbon Paste Electrodes. *Molecules* **16** (2011), 1349–1365. https://doi.org/10.3390/molecules16021349
12. Sohrabi M., Ahti T., Urbanavichus G. Parmelioid Lichens of Iran and the Caucasus Region. *Mycology Balcanica* **4** (2007), 21–30
13. Crawford S.D. Lichens Used in Traditional Medicine. In: Ranković B. (ed.) *Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential*. Springer, Cham (2019), 31–97. https://doi.org/10.1007/978-3-030-16814-8_2
14. Kosanić M., Ranković B. Lichen Secondary Metabolites as Potential Antibiotic Agents. In: Ranković B. (ed.) *Lichen Uecondary Uetabolites: Bioactive Properties and Pharmaceutical Potential* (2nd ed.). Springer, Cham (2019), 99–127. https://doi.org/10.1007/978-3-030-16814-8_3
15. Karagöz A., Doğruöz N., Zeybek Z., Aslan A. Antibacterial Activity of Some Lichen Extracts. *J. Med. Plants Res.* **3** (2009), 1034–1039.
16. Kinoshita K., Matsubara H., et al. Topics in the Chemistry of Lichen Compounds. *J. Hattori Bot. Lab.* **76** (1994), 227–233. https://doi.org/10.18968/jhbl.76.0_227
17. Mitrović T., Stamenković S., et al. Antioxidant, Antimicrobial and Antiproliferative Activities of Five Lichen Species. *Int. J. Mol. Sci.* **12** (2011), 5428–5448. https://doi.org/10.3390/ijms12085428

18. Maciag-Dorszyńska M., Wegrzyn G., Guzow-Krzemińska B. Antibacterial Activity of Lichen Secondary Metabolite Usnic Acid Is Primarily Caused by Inhibition of RNA and DNA Synthesis. *FEMS Microbiol. Lett.* **353** (2014), 57–62. https://doi.org/10.1111/1574-6968.12409
19. Kinalioğlu K., Aydin S., İlhan Ö., Yilmaz Z. Phenol and Flavonoid Amounts, Antioxidant and Antibacterial Potencies of *Xanthoparmelia stenophylla* (Ach.) Ahti&D. Hawksw. *AKU J. Sci. Eng.* **16** (2016), 6–15. https://doi.org/10.5578/fmbd.22064
20. Karaahmet Z., Kinalioğlu K., Aydin S. Antioxidant and Antibacterial Potencies of *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale and *Dermatocarpon miniatum* (L.) W. Mann. Lichens from Black Sea Region in Turkey. *Gümüşhane Univ. J. Sci. Tech. Inst.* **9** (2019), 415–424. https://doi.org/10.17714/gumusfenbil.462040
21. Kumar J., Dhar P., Tayade A.B., et al. Antioxidant Capacities, Phenolic Profile and Cytotoxic Effects of Saxicolous Lichens from Trans-Himalayan Cold Desert of Ladakh. *PLoS One* **9** (2014), Article No: e98696, 19 p. https://doi.org/10.1371/journal.pone.0098696

Ա. Գ. ՍԻՄՈՆՅԱՆ, Ռ. Ռ. ՍԱՐԳՍՅԱՆ, Հ. Հ. ՓԱՆՈՍՅԱՆ, Ա. Հ. ԹՌՉՈՒՆՅԱՆ

XANTHOPARMELIA STENOPHYLLA ԷՊԻԼԻԹԱՅԻՆ ՔԱՐԱՔՈՍԻ ՀԱԿԱԲԱԿՏԵՐԻԱԿԱՆ ԵՎ ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ԱԿՏԻՎՈՒԹՅՈՒՆՆԵՐԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Ուսումնասիրվել է Նորաշենից (Գեղարքունիքի մարզ, ՀՀ) հավաքված էպիլիթային քարաքոս *Xanthoparmelia stenophylla*-ի տարբեր լուծամզվածքների հակաբակտերիական և հակաօքսիդանտային հատկությունները: Քարաքոսային թալոմի մեթանոլային, էթանոլային և ացետոնային լուծամզվածքները դրսևորել են հակաբակտերիական ակտիվություն միայն տեստավորված Գրամ-դրական բակտերիաների նկատմամբ: Մեթանոլային լուծամզվածքը ցուցաբերել է ԴՊՊՀ-ի ռադիկալների չեզոքացման առավելագույն ակտիվություն (68%). Ջրային լուծամզվածքը չի դրսևորել նշանակալի հակաբակտերիական և հակաօքսիդանտային ակտիվություն:

А. Г. СИМОНЯН, Р. Р. САРГСЯН, О. А. ПАНОСЯН, А. А. ТРЧУНЯН

ИЗУЧЕНИЕ АНТИБАКТЕРИАЛЬНОЙ И АНТИОКСИДАНТНОЙ АКТИВНОСТИ ЭПИЛИТНОГО ЛИШАЙНИКА *XANTHOPARMELIA STENOPHYLLA*

Изучена антибактериальная и антиоксидантная активность различных экстрактов эпилитного лишайника *Xanthoparmelia stenophylla*, собранного в Норашене Гегаркуникской области Армении. Метанольные, этанольные и ацетоновые экстракты талома лишайника демонстрировали антибактериальную активность только против протестированных грамположительных бактерий. Метанольный экстракт лишайника показал наибольшую активность по удалению радикалов DPPH (68%). Наши исследования не выявили значительной антибактериальной и антиоксидантной активности водного экстракта.