International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2021.606.610

Research Article
Antibacterial and Enzyme Inhibition Capacities of Peruvian Lichens: *Xanthoparmelia tasmanica* and *Flavopunctelia flaventior*

[1]María del Pilar Caramantin Soriano, [2]Diana Judith Martino-Cruz, [2]Elena Alicia Cóndor-Cuyubamba, [3]Olaf De León-Palomo and [3]Ezequiel Viveros-Valdez

[1]University of Talca, Chemistry Institute of Natural Resources, Av. Lircay S/N, Talca, Chile
[2]National University of Engineering, Faculty of Sciences, School of Chemistry, Av. Túpac Amaru S/N, Rímac, Lima, Perú
[3]Autonomous University of Nuevo León, Faculty of Biological Sciences, Pedro de Alba, Cd. Universitaria, San Nicolás de los Garza, Nuevo León, México

Abstract

Background and Objective: Lichens are widely used in the traditional medicine of various cultures, highlighting the systematic use of these by pre-Columbian cultures of South America. In this context, the present study intended to describe the antimicrobial activity against Gram-positive and Gram-negative bacteria in addition to *in vitro* enzymatic inhibition by extracts from the Peruvian lichens *Xanthoparmelia tasmanica* and *Flavopunctelia flaventior*. **Methodology:** The antibacterial activity was evaluated by the broth microdilution method to determinate the Minimum Inhibitory Concentrations (MIC) against *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* as well as their inhibitory effect on α-glucosidase and α-amylase. **Results:** The extracts induced a powerful biocidal effect against Gram-positive bacteria, the most susceptible bacterial strain was *Micrococcus luteus* (MIC = 6.25 μg mL^{-1}). The extract of *F. flaventior* showed the highest effect on the inhibition of the α-glucosidase and α-amylase with an IC_{50} of 0.34±0.09 and 0.57±0.17 mg mL^{-1}, respectively, the amylase inhibition was better than that produced by acarbose (0.97±0.08 mg mL^{-1}). **Conclusion:** Current results demonstrated the potent antibacterial activity of lichen in extracts from *X. tasmanica* and *F. flaventior*, besides, inhibit carbohydrate digestive enzymes. These properties may be utilized to treat postprandial hyperglycemia and regulate glycemia in diabetic patients.

Key words: Bioassay, bactericidal, enzyme inhibition, lichens, glycemia

Citation: del Pilar Caramantin Soriano, M., M.D.J. Martino-Cruz, E.A. Cóndor-Cuyubamba, O. De León-Palomo and E. Viveros-Valdez, 2021. Antibacterial and enzyme inhibition capacities of Peruvian lichens: *Xanthoparmelia tasmanica* and *Flavopunctelia flaventior*. Int. J. Pharmacol., 17: 606-610.

Corresponding Author: Ezequiel Viveros-Valdez, Ezequiel Viveros-Valdez, Autonomous University of Nuevo León, San Nicolás de los Garza, Nuevo León, Mexico Tel: 52+(81)- 8329 4110

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lichens have been considered as a stable symbiotic association between a fungus and algae and/or cyanobacteria, also contain basidiomycete yeasts, that likely produces chemicals that help lichens to protects them against predators and harmful microorganisms[1]. The lichens feature many properties, like drug and chemical production, which makes them interesting for research and lichens are also important to study them in the ecological context, like food supply for animals[2]. All the potential applications mentioned above are related to its complex structure and organization that results in an abundant chemical diversity such as lecanoric acid, salazinic acid, sekikaic acid, usnic acid, gyrophoric acid, lobaric acid and others[1-3]. According to the systematic study of pharmacological properties, the lichen compounds show antimicrobial, antitumor, anti-inflammatory, analgesic, allergenic, antibiotic and other biological activity[3-5]. In countries such as Austria, China, Italy, India, Indonesia, Germany, Mexico, Russia, Slovakia, Spain and Turkey, usnic acid is used in the medicinal field for the treatment of various ailments, including fractures, skin rashes, throat inflammations, dental pain, respiratory diseases, diuretic, menstrual control, urinary disorders, among others[6,7].

Since their discovery, antibiotics have saved millions of lives. However, the broad use of these drugs has caused a significant increment in bacterial resistance[8]. A bacterial strain can develop several resistance mechanisms against one or several antibiotics so that resistance to multiple substances is a public health problem that has been observing worldwide in recent years[9]. Only in the United States that antibiotic resistance had contributed to the illness of at least two million and 23,000 deaths[10]. For decades, natural products have played a key role in the advancement of novel antibiotic compounds, the unique and chemical diversity of the defence strategies adopted by slow-growing lichens against microorganism attacks may show beneficial medical applications that could be clinically significant against infections[11]. The antimicrobial effect of different lichens like *Cladonia furcata*, *Ochrolechia androgyna*, *Parmelia caperata* and *Parmelia conspersa* has already been demonstrated, which has been attributed to the presence of fumarprotocetraric acid, lecanoric acid, protocetraric acid and stictic acid[12].

On the other hand, Diabetes Mellitus (DM) is a chronic disease of multiple causes, which has become an epidemic in this century and a challenge for the World Health Organization (WHO), the number of people living with diabetes in the world is approximately 451 M, causing a high number of deaths per year[13]. China is in the first position regarding the number of cases, however, the prevalence of diabetes stood at around 11% in 2019, below countries such as Germany or Mexico, where more than 15% of the adult population suffered from this disease[14]. Diabetes is still an incurable disease. But there are a variety of treatments and behavioural changes that are used to control sugar levels in the body. Examples include the use of insulin, medications such as glibenclamide, changes in diet and an exercise routine. Blocking the activity of α-glucosidase is one of the most common therapeutic targets for the development of new drugs to treat type II diabetes[15]. It has been reported that α-glucosidase inhibitors control the release of insulin, which leads to a decrease in lipids, so they have been proposed to treat a wide range of diseases, including lysosomal disorders, certain types of cancers, antiviral, fungistatic and other[16]. Another of the key enzymes in carbohydrate metabolism is α-amylase since it initiates the digestion process, by hydrolyzing the starch and/or glycogen in maltose and ultimately glycemia[17].

Aiming for the most effective therapeutic approaches in the reduction of plasma glucose levels and consequently, suppression of postprandial hyperglycemia, several lichen extracts and isolated compounds have been studied as inhibitors of enzymes (α-amylase and α-glucosidase)[4,17]. Extracts from lichens were previously evaluated as potential inhibitors of α-amylase and/or α-glucosidase[4,18,19]. In addition to them, four extracts of the lichens *Ramalina celastri*, *R. nervulosa* and *R. pacifica* have been used in the inhibition of α and β-glucosidase, demonstrating a promising anti hypoglycemic effect. The activity was associated with lichen metabolites salazinic acid, sekikaic acid and usnic acid[20].

Lichens have been used in traditional medicine across the world, like India and the pre-Columbian cultures of South America. In the last decades, lichens have been investigated regarding the pharmacological properties of their secondary metabolites[21].

In the present work, the acetonic extracts from the lichens *Xanthoparmelia tasmanica* and *Flavopunctelia flaventior*, collected in the Peruvian Andes were obtained and subjected to antibacterial assays. In addition, the α-glucosidase and α-amylase inhibition induced by these extracts were also evaluated.

MATERIALS AND METHODS

Study area: The lichen species *Xanthoparmelia tasmanica* (Hook. f. Taylor) and *Flavopunctelia flaventior* (Stirt.) Hale was collected in May-June, 2014 in the Peruvian Andes (Jauja Province, Junín region, 3200 m at sea level). The lichens

samples were found in different types of microhabitats. *X. tasmanica* was found on stones whereas *F. flaventior* covers surfaces of the tree trunk. The lichens were identified and classified by Biol. Angel Ramirez, taxonomist of the Natural History Museum from the Major National University of San Marcos, Perú.

Preparation of lichen extracts: The lichen samples were dried at 40°C, ground to a uniform powder. *X. tasmanica* (50 g) was extracted by maceration with acetone (3×24 hrs×100 mL), the extracts were combined and concentrated under reduced pressure to yield 4.2% (2.1 g) of crude extract. The lichen *F. flaventior* (63.47 g) was extracted by maceration with acetone (4×48 hrs×150 mL), the extracts were mixed and concentrated under reduced pressure to obtain 10.9% (6.92 g) of crude extract. The organic extracts were kept at 4°C until their bioassays were realized.

Antibacterial activity: Clinical isolates of Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Micrococcus luteus*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*) were tested. By using cultures of the above-mentioned microorganisms, the bacterial suspension (after 18-24 hrs) was pre-pared and turbidity was adjusted to 0.5 in the McFarland standard, which corresponded to 1.5×10^8 colony forming units (CFU mL^{-1})[22].

The Minimum Inhibitory Concentration (MIC) was determined in 96-well microplates by the broth microdilution method. Samples were dissolved in Mueller-Hinton medium (10% DMSO) and 100 µL were added per well, making serial dilutions of each extract (200-0.8 µg mL^{-1}) and positive control (16-0.063 µg mL^{-1}). Ten µL of inoculum of each microorganism was added to each well. Plates were incubated for 24 hrs at 37°C and 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added. Controls were gentamicin and DMSO (1%) as a positive and negative control, respectively. The MIC value is the lowest concentration of a sample at which no colour change of MTT was observed. The tests are carried out in triplicate.

Enzymatic inhibition assays: The enzymatic inhibition activity was evaluated according to the chromogenic methods described in the literature[23]. After a pre-incubation at 37°C for 15 min, a mixture of 1:1 of sample and α-glucosidase (0.8 U mL^{-1}) were incubated in 96 well plates at 37°C for 15 min. After that, a volume of 625 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) solution was added to each well and incubated for another 15 min. Then, the reaction was stopped by adding 100 µL of 0.2 M $NaCO_3$ into each well and the absorbance reading was recorded at 405 nm. For the α-amylase inhibitory activity assay, 50 µL of sample and 50 µL of α-amylase (1 U mL^{-1}) were incubated in 96 well plates at 37°C for 15 min. After that, 50 µL of 0.5% starch solution in phosphate buffer was added to each well and the reaction was incubated at 37°C for 20 min. The reaction was stopped with 20 µL of 1M HCl, followed by the addition of 50 µL of iodine reagent and absorbance was read at 750 nm. The DMSO (<5%) was used as a solvent for pharmacological substances and acarbose as a positive control in both assays. The tests were performed in triplicate and the information is reported as mean±Standard Deviation (SD). The half-maximum inhibitory concentration (IC_{50}) values were determined using probit regression with the SPSS version 17.0 software. The data were compared by using the Student's t-test and Bonferroni multiple comparison post-test, considering $p<0.01$ as significant.

RESULTS

The antibacterial effect of acetonic extracts from *X. tasmanica* and *F. flaventior* are showed in Table 1. Both the extracts were highly efficient against all the Gram-positive bacteria tested. The most sensitive strains were *M. luteus* and *B. cereus* with MIC between 6.25 and 12.5 µg mL^{-1}, respectively. No inhibition against Gram-negative bacteria was observed at the doses used. Dimethyl Sulfoxide (DMSO) was used as a negative control, which offered no interference with the results obtained in the assays.

The inhibitory effect of the analyzed acetonic extracts from lichens are presented in Table 2. The extract of *F. flaventior* showed the highest inhibition efficiency against the α-glucosidase and α-amylase with an IC_{50} of 0.34±0.09 and 0.57±0.17 mg mL^{-1}, respectively. The extract of *F. flaventior* showed the highest inhibitory activity compared to the positive control used, this being 2-5 times more effective than *X. tasmanica*.

Table 1: Antibacterial activity of acetonic extracts from *X. tasmanica* and *F. flaventior*

	Minimum inhibitory concentration (µg mL^{-1})		
Strain	*X. tasmanica*	*F. flaventior*	Gentamicin
S. aureus	25	12.5	0.5
E. faecalis	>200	50	2
L. monocytogenes	>200	100	2
B. cereus	12.5	12.5	1
M. luteus	6.25	6.25	0.5
E. coli	>200	>200	1
S. typhimurium	>200	>200	2
P. aeruginosa	>200	>200	2

n = 3

Table 2: Enzymatic inhibition of acetonic extracts from *X. tasmanica* and *F. flaventior*

	Average inhibitory concentration (mg mL^{-1})	
Sample	α-Glucosidase	α-Amylase
X. tasmanica	0.75±0.23[a]	2.20±0.68[a]
F. flaventior	0.34±0.09[b]	0.57±0.17[b]
Acarbose	0.12±0.20[c]	0.97±0.08[c]

n = 3, p<0.01, superscript alphabets showed different significance level

DISCUSSION

In the present research, the biological activity of the acetonic extracts of lichens *X. tasmanica* and *F. flaventior* collected in Peru was determined. Both lichen species belong to the family Parmeliaceae, one of the most studied in the world[24]. The research focused on the use of these lichens in traditional medicine, which includes the treatment of infectious and metabolic diseases.

According to the results obtained in this work, *X. tasmanica* and *F. flaventior* have a powerful biocidal effect on Gram-positive bacteria, being *M. luteus* and *B. cereus* the most sensitive strains to both extracts, showing a MIC of 6.25 and 12.5 μg mL^{-1}, respectively. The observed bactericidal effect was most evident for Gram-positive bacteria. This result can be attributed to the fact that the Gram-negative bacteria present two lipid membranes between each other which a peptidoglycan cell wall is located. This outer membrane protects the bacteria from several antibiotics that would normally damage the internal membrane or the cell wall[25]. The experimental data of the present work corroborates the previously published reporting the antimicrobial effect of lichens of the genus Xanthoparmelia on *S. aureus* and *B. cereus*[26] and *F. flaventior* against *S. aureus* and *K. pneumoniae*[27].

The α-glucosidase is a key enzyme for the regulation of plasma glucose levels allowing to prevent pathologies of great importance among them diabetes mellitus. The inhibitory effect of acetonic extracts from *X. tasmanica* and *F. flaventior* on α-glucosidase are presented in Table 2. The effect of *F. flaventior* extract was close to that shown by acarbose (0.12±0.02 mg mL^{-1}). The powerful effect of other lichen extracts has already been previously reported and it was attributed to the presence of zeorine, methyl β-orcinol carboxylate and methyl orsellinate[4,28]. The blood glucose level may rise in part due to the role of α-amylase activity. Therefore, the inhibition of this enzyme can cause a decrease in postprandial hyperglycemia and could be used as a potential strategy in the treatment of diabetes mellitus[17].

Acetonic extracts of studied species exhibited strong α-amylase inhibitory effects. *F. flaventior* display greater effect than that obtained for acarbose (0.97±0.08 mg mL^{-1}), used as referential control. The inhibitory effect of extracts from other lichens species on the α-amylase has already been reported, the inhibitory activity was attributed to the presence of usnic, salazinic and lecanoric acids[4,18,19].

The results obtained in this research support the use of both lichens in traditional Peruvian medicine, however, further research is required to scientifically confirm and support their use as complementary and/or substitute medicine.

CONCLUSION

The lichens exhibit pharmacological properties, such as antimicrobial, antitumor, anti-inflammatory, analgesic, allergenic, antibiotic and other biological activities. Here the antibiotic and anti-enzymatic activity of Peruvian lichens extracts were demonstrated, promising results regarding the utilization against Gram-positive bacteria and the enzymatic inhibition of the α-glucosidase and α-amylase, which are involved in the glycemic control, with *F. flaventior* extract being the most active. It is the first report where the potential of *X. tasmanica* and *F. flaventior* as enzymatic inhibitors is addressed, which could be promising sources of new agents to treat diabetes mellitus.

SIGNIFICANCE STATEMENT

The present study reports the bactericidal and enzymatic inhibition of acetonic extract from of *X. tasmanica* and *F. flaventior* collected in Peru, which are used in traditional medicine. It aimed to expand upon the already existing knowledge regarding this species, concurrently proposing the metabolic prospecting and further characterization of candidate compounds for pharmacological uses. Additionally, it provides a preliminary validation of traditional uses.

ACKNOWLEDGMENT

The authors acknowledge PAICYT-UANL: CN1581-21 for the financial support. We are grateful to Rachael Jiménez-Lange, Language Program of Universidad de Talca for revising the English version of the manuscript.

REFERENCES

1. Spribille, T., V. Tuovinen, P. Resl, D. Vanderpool and H. Wolinski *et al*., 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. Science, 353: 488-492.
2. Allen, J.L., R.T. McMullin, E.A. Tripp and J.C. Lendemer, 2019. Lichen conservation in North America: A review of current practices and research in Canada and the United States. Biodivers. Conserv., 28: 3103-3138.
3. Molnar, K. and E. Farkas, 2010. Current results on biological activities of lichen secondary metabolites: A review. Zeitschrift fur Naturforschung C, 65: 157-173.
4. Thadhani, V.M. and V. Karunaratne, 2017. Potential of lichen compounds as antidiabetic agents with antioxidative properties: A review. Oxid. Med. Cell. Longevity, Vol. 2017. 10.1155/2017/2079697.
5. Solárová, Z., A. Liskova, M. Samec, P. Kubatka, D. Büsselberg and P. Solár, 2020. Anticancer potential of lichens' secondary metabolites. Biomolecules, Vol. 10. 10.3390/biom10010087.
6. Cocchietto, M., N. Skert, P. Nimis and G. Sava, 2002. A review on usnic acid, an interesting natural compound. Naturwissenschaften, 89: 137-146.
7. Zugic, A., V. Tadic and S. Savic, 2020. Nano- and microcarriers as drug delivery systems for usnic acid: Review of literature. Pharmaceutics, Vol. 156. 10.3390/pharmaceutics12020156.
8. Alós, J.I., 2015. Antibiotic resistance: A global crisis. Enfermeda Infecciosas Microbiología Clín., 33: 692-699.
9. Giedraitiene, A., A. Vitkauskiene, R. Naginiene and A. Pavilonis, 2011. Antibiotic resistance mechanisms of clinically important bacteria. Medicina, Vol. 47, No. 3. 10.3390/medicina47030019.
10. Solomon, S.L. and K.B. Oliver, 2014. Antibiotic resistance threats in the United States: Stepping back from the brink. Am. family Physician., 89: 939-941C.
11. Muller, K., 2001. Pharmaceutically relevant metabolites from lichens. Applied Microbiol. Biotechnol., 56: 9-16.
12. Ranković, B. and M. Mišić, 2008. The antimicrobial activity of the lichen substances of the lichens *Cladonia furcata*, *Ochrolechia androgyna*, *Parmelia caperata* and *Parmelia conspresa*. Biotechnol. Biotechnol. Equip., 10.1080/13102818.2008.10817601.
13. Cho, N., J.E. Shaw, S. Karuranga, Y. Huang, J.D. da Rocha Fernandes, A.W. Ohlrogge and B. Malanda, 2018. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract., 138: 271-281.
14. Saeedi, P., I. Petersohn, P. Salpea, B. Malanda and S. Karuranga *et al*., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9th edition. Diabetes Res. Clin. Pract., Vol. 157. 10.1016/j.diabres.2019.107843
15. Raskin, P.R., P.A. Hollander, A. Lewin, R.A. Gabbay, B. Bode and A.J. Garber, 2007. Basal insulin or premix analogue therapy in type 2 diabetes patients. Eur. J. Internal Med., 18: 56-62.
16. Asano, N., 2003. Glycosidase inhibitors: Update and perspectives on practical use. Glycobiology, 13: 93R-104.
17. Tundis, R., M.R. Loizzo and F. Menichini, 2010. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. Mini Rev. Med. Chem., 10: 315-331.
18. Kekuda, T.R.P., D. Lavanya and R. Pooja, 2019. Lichens as promising resources of enzyme inhibitors: A review. J. Drug Delivery Ther., 9: 665-676.
19. Vinayaka, K.S., S. Karthik, K.C. Nandini and P.T.R. Kekuda, 2013. Amylase inhibitory activity of some macrolichens of Western Ghats, Karnataka, India. Indian J. Novel Drug Delivery, 5: 225-228.
20. Verma, N., B.C. Behera and B.O. Sharma, 2012. Glucosidase inhibitory and radical scavenging properties of lichen metabolites salazinic acid, sekikaic acid and usnic acid. Hacettepe J. Biol. Chem., 40: 7-21.
21. Rodríguez, Rodríguez, E.F., A.M.R. Ordaya, E.A. Izquierdo, L.P. Velásquez, S.L. González and R.A. Tocas, 2017. Catalogue of the lichenobiota of La Libertad region, Peru. Arnaldoa, 24: 497-522.
22. Viveros-Valdez, E., C. Rivas-Morales, A. Oranday-Cardenas, M.J. Verde-Star and P. Carranza-Rosales, 2011. Antimicrobial activity of *Hedeoma drummondii* against opportunistic pathogens. Pak. J. Biol. Sci., 14: 305-307.
23. Garcia-Davis, S., M. Munoz-Ochoa, C. Rivas-Morales and E. Viveros-Valdez, 2018. Biological activities from the marine sponge *Suberites aurantiacus*. J. Biol. Sci., 18: 152-157.
24. Gómez-Serranillos, M.P., C. Fernández-Moriano, E. González-Burgos, P.K. Divakar and A. Crespo, 2014. Parmeliaceae family: Phytochemistry, pharmacological potential and phylogenetic features. RSC Adv., 4: 59017-59047.
25. Zhang, G., T.C. Meredith and D. Kahne, 2013. On the essentiality of lipopolysaccharide to gram-negative bacteria. Curr. Opin. Microbiol., 16: 779-785.
26. Candan, M., M. Yılmaz, T. Tay, M. Kıvança and H. Türk, 2006. Antimicrobial activity of extracts of the lichen xanthoparmelia pokornyi and its gyrophoric and stenosporic acid constituents. Z. Naturforsch., 61: 319-323.
27. Rodríguez, O.E.A, W.A.B. Andrade, F.E.L. Díaz and B. Moncada, 2015. Actividad antimicrobiana de líquenes de la cuenca alta del rio Bogotá. Nova, 13: 47-64.
28. Karunaratne, V., V.M. Thadhani, S.N. Khan and M.I. Choudhary, 2014. Potent α-glucosidase inhibitors from the lichen *Cladonia* species from Sri Lanka. J. Nat. Sci. Found. Sri Lanka, 42: 95-98.