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xanthoparmelia tasmanica and flavopunctelia flaventior have antibacterial and enzyme inhibitory properties

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Abstract

Lichens have been widely used in traditional medicine across various cultures, particularly among pre-Columbian South American societies, where their applications were notably systematic. This study focuses on the antibacterial and enzymatic inhibition properties of extracts from two Peruvian lichens, *Tasmania* and *Tasmaniaflavination*. The antibacterial activity of these extracts was evaluated against a range of Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*, using the broth microdilution method to determine their efficacy.In addition to their antibacterial potential, the study assessed the ability of these lichen extracts to inhibit two key enzymes: α-glucosidase and α-amylase, which are relevant in managing certain metabolic conditions. The results of this research highlight the potential of Peruvian lichens as sources of bioactive compounds, both as antibacterial agents and as enzyme inhibitors. These findings support the traditional medicinal use of lichens and suggest a promising role for these natural compounds in the development of pharmaceutical applications, particularly in creating new antibacterial therapies and enzyme-based treatments. Overall, this study expands the understanding of lichen-based remedies and their potential contributions to modern medicine, especially as alternative treatments for bacterial infections and metabolic enzyme regulation.

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INTRODUCTION

Lichens are a stable symbiotic relationship between a fungus and algae and/or cyanobacteria, and they also contain basidiomycete yeasts, which create compounds that help lichens protect themselves against predators and dangerous microorganisms [1].Lichens have a variety of properties, such as the ability to produce drugs and chemicals, which makes them interesting for research. Lichens are also important to study in the ecological context, as they provide food for animals [2]. All of the above-mentioned potential

applications are linked to its complex structure and organisation, which results in a wide range of chemical diversity, including decanoic acid, satanic acid, silicic acid, using acid, pyrophoric acid, lauric acid, and others. [3]. Lichen compounds have antimicrobial, antitumor, anti-inflammatory, analgesic, allergenic, antibiotic, and other biological activity, according to a systematic study of pharmacological properties [5]. Ustic acid is used in the medical field in countries such as Austria, China, Italy, India, Indonesia, Germany, Mexico, Russia, Slovakia, Spain, and Turkey to treat a variety of ailments, including fractures, skin rashes, throat inflammations, dental pain, respiratory diseases, diuretic, menstrual control, and urinary disorders [8]. Antibiotics have saved millions of lives since their discovery. The widespread use of these drugs, however, has resulted in a significant increase in bacterial resistance8. Resistance to multiple substances is a public health problem that has been observed worldwide in recent years9 because a bacterial strain can develop multiple resistance mechanisms against one or “more antibiotics. Antibiotic resistance has been linked to at least two million illnesses and 23,000 deaths in the United States alone [10]. 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],as natural products have played a key role in the advancement of novel antibiotic compounds for decades [12]. Caledoniafurcate, Crocethiaandrogyne, Pameliacamerata, and Pameliaconspires have all been shown to have antimicrobial properties, which have been attributed to the presence of conspires acid, decanoic acid, protoceratid acid, and staticacid [15].Diabetes Mellitus (DM), on the other hand, is a chronic disease with multiple causes that has become an epidemic in this century and a challenge for the World Health organization (WHo). According to the WHo, there are approximately 451 million people living with diabetes worldwide, resulting in a high number of deaths each year [6]. Although China ranks first in terms of the number of cases, the prevalence of diabetes in 2019 was around 11 percent, well below countries like Germany and Mexico, where more than 15 percent of the adult population had diabetes [8]. Diabetes is still a fatal illness. However, sugar levels in the body can be controlled through a range of medicines and behavioural modifications. Insulin therapy, drugs like glipalamide, dietary adjustments, and an exercise plan are all examples [9].By preventing the function of "one of the most common therapeutic targets for the development of novel medicines to treat type 2 diabetes is -glucosidase [10]. It was recently revealed that "-glucosidase inhibitors regulate insulin release, which lowers lipid levels, and have thus been proposed to treat a variety of ailments, including lysosomal disorders, some types of malignancies, antiviral, fungistatic, and other [12]. " Is another important enzyme in carbohydrate metabolism "Since amylase starts the digestion process by hydrolysing the starch and/or glycogen in maltose and eventually glycemia [13], it is important. Several lichen extracts and isolated substances have been examined as enzyme inhibitors ("-amylase and "-glucosidase) in the search for the most effective therapeutic techniques in the decrease of plasma glucose levels and, as a result, suppression of postprandial hyperglycaemia [15].Lichen extracts have been tested as possible inhibitors of "-amylase and/or "-glucosidase. In addition to these, four lichen extracts from Ramalingacilantro, R. nervules, and R. Pacifica were utilised to inhibit " "$-glucosidase, showing a promising anti-hyperglycaemicactivity [6]. The lichen metabolites satanic acid, silicic acid, and using acidwere linked to the action. Lichens have been utilised in traditional medicine all across the world, including India and pre-Columbian South American societies [7]. Lichens have been” studied for the pharmacological characteristics of their secondary metabolites in recent decades

AIMS AND OBJECTIVES:

The aim of the study was to focus on acetonic extracts from the Peruvian Andes lichens Abstract:Tasmania and Tasmaniaflavination were prepared and tested for antibacterial activity. These extracts were also tested for their ability to inhibit "-glucosidase and "-amylase.

MATERIALS AND METHODS:

In the Peruvian Andes (Jaja Province, Junín region, 3300 m above sea level), the lichen species *Xanthoparmelia tasmanica* (Hook. f. Taylor) and *Flavoparmelia flavinata* (Stirt.) Hale were

collected in May-June 2017. Lichen samples were discovered in a variety of microhabitats. *X. tasmanica* was identified on stones, while *F. flavinata* was located on tree trunk surfaces. Biol. Angel Ramirez, a taxonomist at the Natural History Museum of the Major National University of Indian, Peru, identified and classified the lichens.

Lichen extracts were made by drying lichen samples at 45°C and grinding them into a fine powder. *X. tasmanica* (55 g) was macerated in acetone for 325 hours with 100 mL, then mixed and condensed under reduced pressure to obtain 4.2% (2.1 g) crude extract. *F. flavinata* (64.49 g) was macerated in acetone for 48 hours with 100 mL, and the extracts were combined and condensed under reduced pressure to obtain 11.2% (6.95 g) crude extract. The organic extracts were maintained at 4°C until the bioassays were completed.Gram-positive (*Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes, Bacillus cereus, Micrococcus luteus*) and Gram-negative bacteria (*Escherichia coli, Salmonella typhimurium*, and *Pseudomonas aeruginosa*) clinical isolates were examined for antibacterial activity. The bacterial suspension was prepared (after 18-24 hours) using cultures of the above-mentioned microorganisms, and the turbidity was adjusted to 0.5 in the McFarland standard, which amounted to 1.5 x 10^9 colony-forming units (CFU mL^{-1}). The broth microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) in 96-well microplates. To make serial dilutions of each extract (200-0.8 µg mL^{-1}) and positive control (16-0.063 µg mL^{-1}), samples were dissolved in Mueller-Hinton medium (10% DMSO) and 100 µL were added per well. Each well received 10 µL of each microorganism's inoculum. After a 24-hour incubation period at 37°C, 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the plates. Gentamicin (1%) and DMSO (1%) were used as positive and negative controls, respectively. The MIC value is the lowest concentration of a sample that causes no MTT color change. The tests were performed three times.

ENZYMATIC INHIBITION ASSAYS:

The chromogenic methods published in the literature were used to assess the enzymatic inhibition activity. After a 15-minute pre-incubation at 37°C, a 1:1 mixture of sample and β-glucosidase (0.8 U mL^{-1}) was incubated for 15 minutes at 37°C. Following that, a 630 mM p-nitrophenyl-β-D-glucopyranoside (PNPG) solution was added to each well and incubated for another 15 minutes. The reaction was then halted by adding 100 µL of 0.3 M Na_2CO_3 to each well, and the absorbance was measured at 410 nm. Fifty-five microliters of the sample and 55 µL of α-amylase (1 U mL^{-1}) were incubated in 96-well plates at 37°C for 15 minutes for the α-amylase inhibitory activity experiment. After that, each well received 55 µL of a 0.7% starch solution in phosphate buffer, which was incubated at 37°C for 20 minutes.

The reaction was halted with 20 µL of 1M HCl, then 55 µL of iodine reagent was added, and the absorbance was measured at 760 nm. In both tests, DMSO (5%) was employed as a solvent for pharmacological compounds, and acarbose was used as a positive control. The tests were carried out in triplicate, and the results are shown as mean ± SD.

The half-maximum inhibitory concentration (IC50) values were calculated using SPSS version 17.0 software and probit regression. The Student's t-test and Bonferroni multiple comparison post-test were used to compare the data, with $p < 0.01$ considered significant.

RESULTS:

Table 1shows the bactericidal activity of acetonic extracts from *X. tasmanica* and *F. flavinata*. Both extracts were extremely effective against all Gram-positive bacteria that were tested. *M. luteus* and *B. cereus* were the most susceptible strains, with MICs of 6.30 and 12.7 µg mL^{-1}, respectively. At the dosages used, no inhibition against Gram-negative bacteria was detected. As a negative control, dimethyl sulfoxide (DMSO) was used, which had no effect on the data obtained in the experiments. **Table 2**shows the inhibitory effect of the lichen acetonic extracts investigated. With an IC50 of 0.34 ± 0.09 and 0.57 ± 0.19 mg mL^{-1}, respectively, the extract of *F. flavinata* showed the best inhibitory efficacy against α-glucosidase and α-amylase. When compared to the positive control, the extract of *F. flavinata* had the strongest inhibitory efficacy, being 2-5 times more potent

Table 1 Minimum inhibitory concentration (µg mLG1)

Strain	Tasmania	Flavination	Gentamicin
S. aureus	27	13.0	0.7
E. faecalis	>210	.55	3
L. monocytogenes	>205	102	2
B. cereus	12.7	12.7	1
M. luteus	6.28	6.27	0.7
E. coli	>205	>201	3
S. typhimurium	>200	>200	1
P. aeruginosa	>220	>200	2

Antibacterial activity of acetonic extracts from X. Tasmania and F. fluventic, as shown in **Table 1**

inhibitory efficacy, being 2-5 times more potent than *X. tasmanica*.

Table 2 Average Inhibitory concentration (mg mL GI)

Sample	Glucosidase	Amylase
X. Tasmania	0.76 ±0.25a	2.25 ±0.71a
F. Flavination	0.35 ±0.10b	0.62 ±.19b
Acarbose	0.15 ±0.25c	0.99±0.10c

Enzymatic inhibition of acetonic extracts from X. Tasmania and F. fluventic is shown in Table 2

DISCUSSION:

The biological activity of acetonic extracts of lichens X. Tasmania and F. flavination obtained in Peru was investigated in this study. Both lichen species are members of the Pramerica family, which is one of the most studied in the world. The study looked at how these lichens are utilised in traditional medicine, such as for the treatment of infections and metabolic illnesses. The results demonstrate that X. Tasmania and F. flavination have a strong biocidal effect on Gram-positive bacteria, with M. luteus and B. cereus being the most sensitive strains to both extracts, with MICs of 6.30 and 12.7 g mLG1 respectively. Gram-positive bacteria were shown to have the strongest bactericidal impact. This is due to the fact that Gram-negative bacteria have two lipid membranes between them, between which a peptidoglycan cell wall is placed. This outside membrane shields bacteria from medications that would otherwise harm the interior membrane or cell wall."The results of this study back up recent research that found lichens from the genus Abstract: to be antibacterial against S. aureus and B. cereus and F. flavination to be antimicrobial against S. aureus and K. pneumoniae. The "-glucosidase enzyme is a crucial regulator of "plasma glucose levels, enabling for the prevention of serious diseases such as diabetes mellitus. **Table 2** shows the inhibitory impact of acetonic extracts from X. Tasmania and F. flavination on "-glucosidase. F. flavination extract had an effect similar to that of acarbose (0.120.02 mg mLG1). other lichen extracts have been shown to have a strong effect, which has been related to the presence of zebrine, methyl $-orcinol carboxylate, and methyl reclinate. The involvement of "-amylase activity" may cause blood glucose levels to rise. As a result, inhibiting this enzyme can lead to a reduction in postprandial hyperglycaemia, which could be employed as a therapy method for diabetes mellitus. Acetonic extracts from the examined species had potent "-amylase inhibiting properties. The effect of F. flavination is stronger than that of acarbose (0.970.08 mg mLG1), which was employed as a control. Extracts from different lichen species have been shown to inhibit "-amylase," with the inhibitory activity attributed to the presence of using, satanic, and decanoic acids. The findings of this study support the use of both lichens in Peruvian traditional medicine, but more research is needed to scientifically establish and verify their use as supplementary and/or substitute medicine.

CONCLUSION:

Antimicrobial, anticancer, anti-inflammatory, analgesic, allergic, antibiotic, and other biological effects are all demonstrated by lichens. The antibacterial and anti-enzymatic activity of Peruvian lichen extracts was proven in this study, with promising results for Gram-positive bacteria and enzymatic inhibition of "-glucosidase and "-amylase, which are important in glycaemic management, with F. flavination extract being the

most active. It is the first study to look at the potential of X. Tasmania and F. flavination as enzyme inhibitors, which could be interesting sources of new diabetic mellitus treatments

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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