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ORIGINAL ARTICLE

GC-MS Profiling and Pharmacological Potential of *Physconia venusta* (Ach.) Poelt

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ABSTRACT

Objectives: Lichens are complex symbiotic organisms that generate various bioactive compounds with significant therapeutic value. We investigated the chemical composition and bioactivity of the acetone extract of the Algerian lichen *Physconia venusta* (Ach.) poet.

Materials and Methods: Phytochemical screening was performed using gas chromatography-mass spectrometry (GC-MS). The antibacterial activity was assessed against *Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhi, Staphylococcus aureus, Listeria monocytogenes,* and *Bacillus subtilis* using an agar diffusion test with the determination of the minimal inhibition concentration (MIC), while the antioxidant activity was determined using different chemical methods (DPPH, ABTS, CUPRAC, reducing power, superoxide anion scavenging, β-carotene bleaching, and metal chelate). In addition, cytotoxic activity was tested using *Artemia salina* (Brine shrimp) bioassay.

Results: The studied extract exhibited intense antibacterial activity against *E. coli* and *S. aureus* with inhibition diameters of 28 ± 0.01 and 22 ± 0.01 mm, respectively, with a MIC value of 6.25 mg/mL and a selectivity index of 2.8. The obtained extract showed different antioxidant trends depending on the selected assay. GC-MS analysis revealed many secondary metabolites.

Conclusion: *P. venusta*, a type of lichen, is a potential source of bioactive substances that could be used in pharmaceuticals.

Keywords: *Physconia venusta,* antioxidant, brine shrimp lethality assay, antibacterial, GC-MS, usnic acid

INTRODUCTION

Lichens are symbiotic organisms composed of fungi (mycobiont) and green algae or/and cyanobacteria (photobiont).[1] To treat skin, respiratory, and digestive issues, traditional medicine practices more than 52 lichen species worldwide. More than 1000 compounds with various activities, including anti-inflammatory, anti-proliferative, cytotoxic, and anticancer properties, have been described in previous research.[2] More than 1085 lichen species have been identified in Algeria, 64 of which are indigenous.[3]

Their use as bioindicators of air pollution has received the most scientific attention. However, there have been very few scientific studies on lichen chemistry in this highly diverse country. Antibiotic resistance in bacteria is a major problem affecting public health. It has been increasing for several decades, making it more challenging to treat patients, lengthening the time spent providing care, and increasing infection-related morbidity. Antibiotic resistance has, according to the OMS 2017 report, alarmingly increased on a global scale. The ability to cure widespread infectious diseases is threatened by the emergence and dissemination of new resistance mechanisms.[4] Treatment is becoming more difficult and occasionally impossible for a growing array of infections, including pneumonia, tuberculosis, sepsis, and foodborne

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illnesses, because antibiotics lose their potency. The estimate is that by 2050, an effective antibiotic will only be available if new drugs are developed or discovered.[5] Therefore, there is an urgent need for novel antibacterial agents. Researchers from all around the world have recently paid close attention to the quest for novel antibacterial compounds in medicinal plants.[6] Medicinal plants contain a variety of antibacterial molecules like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinines, and coumarins.[7] Usnic acid is a di-benzofuran compound known as a secondary lichen metabolite. Numerous studies have documented this substance's biological properties, which include antibacterial, anti-inflammatory, antioxidant, antiviral, and antitumoral effects.[8]

This study aimed to investigate the phytochemical profile of *Physconia venusta* (Ach.) Poelt (Kingdom: *Fungi*, phylum: *Ascomycota*, Class: *Lecanoromycetes*, Order: *Teloschistales,* Family: *Physciaceae*, Genus: *Physconia*), (Catalog #: WIS-L-0136263, Occurrence ID: 773fe232-313e-42b9-a4d6-5689e4a6b748, www.lichenportal.org) by measuring its levels and usnic acid content.

One of the rare lichens with a distribution centered in Italy, Morocco, and Algeria is *P. venusta (Ach.)* Poet, whose phytochemicals have not received much attention.[9]

We investigated the antibacterial activity of the obtained extract against seven bacterial strains known for their resistance to antibiotics and multiple nosocomial and chronic infections. Finally, the antioxidant capacity of the same extract was assessed as persistent bacterial infections often associated with a high production of free radicals in the body.

MATERIALS AND METHODS

Lichen collection

A lichen sample of *Physconia venusta* (Figure 1) was collected from Elmeridje forest-Constantine in northeast Algeria. The specimen was identified by Dr. Philippe Clerc (Conservatory and Botanical Garden of Geneva, Chambésy, Switzerland). The sample was dried at room temperature and then ground to obtain a fine powder, which was stored in the dark until extraction.

Preparation of the acetone extract from P. venusta

Acetone has been effectively used to extract antioxidants, including phenolics, because of its chemical properties, such as the capability of dissolving hydrophilic and lipophilic compounds, giving better yields and supporting the biological activities of phytochemicals, avoiding problems with pectins, and allowing lower temperatures for sample preservation.[10,11] The extraction procedure consists of macerating 20 g of powder in 100 mL of acetone at room temperature for 72 hours. The following filtering, the mixture was concentrated in a rotary evaporator (Buchi 23022A120 Rotavapor Cole-Parmer, USA). The obtained extract was stored at 20 °C.

Chemical characterization

Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent according to the method described by Müller et al.[12] In brief, 20 µl of the extract was mixed with 100 µl of Folin-Ciocalteu reagent (diluted at 1:10) and 75 µl of sodium carbonate (7.5%). The mixture was then incubated in the dark for 2 hours, and absorbance was measured at 765 nm. A blank was prepared similarly by replacing the extract with the solvent used.

Total flavonoid content

The total flavonoid content was measured according to the method of Topçu et al.[13] with some modifications for a 96-well microplate assay. Briefly, 50 µl of the extract (1 mg/mL) was mixed with 130 µl of methanol, 10 µl potassium acetate (CH_3COOK), and 10 µl aluminum nitrate [Al $(NO_3)_3$, $9H_2O$]. The mixture was incubated at room temperature for 40 minutes, and absorbance was measured at 415 nm.

Gas chromatography-mass spectrometry (GC-MS) analysis

A Perkin Elmer (Clarus SQ 8C GC/spectrometer, Germany) was used to perform quantitative and qualitative analyses of the chemical composition (gas chromatography linked to mass spectrometry). The oven temperature was initially set to 50 °C and held for 5 minutes. A 30 °C/min ramp was applied up to 270 °C, which was held for an additional 5 minutes. MS spectra were acquired in the electron impact ionization mode, ranging from 50 to 600 a.m.u. Various components were identified by different retention times detected by a mass spectrophotometer. The compounds were identified by comparing the data with existing software libraries like WILEY8.LIB, NIST11.lib and PUBCHEM lib.[14]

Quantification of usnic acid by high-performance liquid chromatography (HPLC)

HPLC analysis was performed according to the method described by Cansaran-Duman et al.[15] All used chemicals were HPLC grade from Sigma-Aldrich, Germany. A 1 mg/mL stock solution of usnic acid was prepared in acetone. All the standards were placed in an autosampler and analyzed. Calibration curves of usnic acid were obtained using seven samples of various concentrations using linear regression analysis (Figure 2).

The analysis was performed on an Agilent 1220 infinity LC system equipped with a diode array detector; a reverse phase C18 column was used. Mobile phase A was a mixture of methanol and phosphate buffer pH 7.4 (70:30 *v/v*) with a flow rate of 0.8 mL/min to detect usnic acid at 245 nm by comparing the retention times with pure standard. For sample analysis, 5 mg of the extract was added to 10 mL of acetone at room temperature; and the mixture was filtered using a 0.45 µm filter. 20 µl of the filtered solution was injected into the HPLC system. All experiments were performed in triplicate.

Antimicrobial activity

Microorganisms and media

The Constantine Research Center in Biotechnology-Algeria provided the bacterial strains including four Gram-negative:

Figure 1. Algerian *Physconia venusta* photographed in the Elmeridje forest-Constantine, Algeria

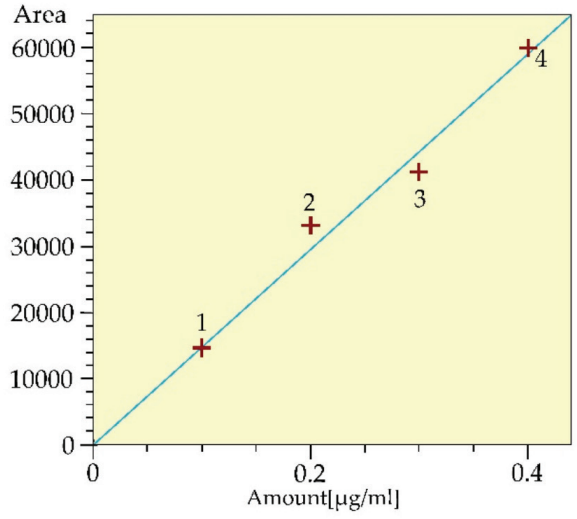

Figure 2. Calibration curve of usnic acid (Sigma); R_2= 0.9896

Escherichia coli (ATCC25922TM), *Pseudomonas aeruginosa* (ATCC27853TM), *Salmonella enteritidis* (ATCC13076TM), *Salmonella typhi* (ATCC14028TM), and four Gram-positive bacteria: *Staphylococcus aureus* (ATCC25923TM), *Listeria monocytogenes* (ATCC 15313TM), *Bacillus* sp. (ATCC16404TM), and *Bacillus subtilis* (ATCC6633TM).

A suspension containing 10^8 colony-forming units (CFU)/mL was prepared from an 18-hour fresh bacterial culture by adjusting the absorbance to 0.1 at 625 nm in physiological water (0, 9%).

Agar disk diffusion

The antimicrobial effect on agar was investigated using the disk diffusion method as previously described.[16] Sterile paper disks of 6-mm size were soaked with acetone extract or usnic acid sigma (15 µl/disk) at two different concentrations (50 mg/mL and 1 mg/mL, respectively) in triplicate and allowed to dry at room temperature under sterile conditions. The plates were then incubated at 37 °C for 24/48 hours. Paper disks loaded with dimethyl sulfoxide (DMSO) were also used as negative controls, whereas gentamicin was used as a positive control. The inhibition zones were reflective of the antimicrobial effectiveness of the extract.

Minimal inhibition concentration (MIC)

MIC was determined by the broth microdilution method using 96-well microtiter plates according to the method described by Londone Bailon et al.[17] For this purpose, a series of dilutions with concentrations ranging from 50 to 0.15 mg/mL and from 1 to 0.0075 mg/mL of the extract and the usnic acid were used in the experiment against every tested strain. 50 µl of a bacterial inoculum (107 CFU/mL) was transferred in each well containing 50 µl of lichen extract or usnic acid solution. After 24 hours of incubation at 37 °C, absorbance was measured at 630 nm, and the MIC was determined. This later corresponded to the lowest concentration, which completely inhibited bacterial growth. A DMSO solution was used as the negative control, and a culture medium with bacteria was used as the positive control. All experiments were performed in duplicate.

Brine shrimp cytotoxicity test

Brine shrimp (*Artemia salina*) eggs (JBL Artemio Mix, Germany) were hatched in seawater (10 g/1L of seawater). The solution was incubated at 28 °C for 48 hours under artificial lighting and aeration provided by an aquarium pump. After incubation, the *nauplii* (larvae) were separated from the remaining eggs and used in the toxicity assay. The effect of the acetone extract on Brine shrimp larvae viability was assessed using the method reported by Sarah et al.[18] Ten larvae of *Artemia* (*nauplii*) were transferred to test tubes containing 100 µL of the extract at different concentrations, where the content was then adjusted to 5 mL by seawater (70%). After 24 hours of incubation, the amount of surviving larvae in each tube was determined. Potassium dichromate was used as the positive control. The percentage of mortality was calculated using the formula below:

$$\%\ death = \frac{(number\ of\ death\ nauplii)}{(total\ number)} \times 100$$

The LC_{50} (lethality of 50% of the larvae) was determined from the regression curve plotted by the mortality rates at different concentrations.

Antimicrobial selectivity index (SI)

The antimicrobial SI is defined as the ratio between the concentrations leading to 50% lysis of cells and the minimum concentration inhibiting bacterial growth (SI = LC_{50}/MIC), which is also indicated as a therapeutic index.[19] SI was calculated using the following equation:

$$SI = LC_{50}\ (mg/mL)/MIC\ (mg/mL)$$

Where LC_{50} refers to the concentration of the sample inducing 50% lethality of *Artemia nauplii* and MIC represents the MIC.

Antioxidant activity

DPPH free radical scavenging assay

The free radical-scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.[20] Briefly, in

a 96-well plate, 160 µL of DPPH (0.1 mM) solution was added to 40 µL of samples at different concentrations (4000, 800, 400, 200, 100, 50, 25, 12.5 µg/mL). The plate was kept in the dark at room temperature for 30 minutes. The absorbance was read at 517 nm. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ascorbic acid were used as standard antioxidants. The scavenging capacity of the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{Ac\text{-}As}{Ac}\right) \times 100$$

Results were given as IC_{50} value (mg/mL) corresponding to the concentration of the sample inducing a 50% reduction of the initial absorbance of DPPH solution.

ABTS assay

The ABTS scavenging activity was evaluated according to the method described by Londone Bailon et al.[17] The ABTS solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium per sulfate for 16 hours. The $ABTS^{+\bullet}$ solution was then diluted in distilled water to an absorbance of 0.708 ± 0.025 at 734 nm. In a 96-well microplate, 40 µL of the sample at different concentrations was mixed with 160 µL of the $ABTS^{+\bullet}$ solution. After 10 minutes of incubation, absorbance was measured at 734 nm. BHA, BHT, and ascorbic acid were used as antioxidant standards. The inhibition percentage was calculated using the equation represented in the DPPH free radical scavenging assay section.

Superoxide anion scavenging activity

The superoxide radical scavenging ability was assessed by measuring the inhibition of O^{2-} generation using alkaline DMSO, as reported by Mazouz et al.[21] The reaction mixture consisted of 40 µL of acetone extract, 130 µL of alkaline DMSO (20 mg NaOH in 100 mL of DMSO), and 30 µL *nitroblue tetrazolium* test solution (1 mg/mL in DMSO). The mixture was incubated at 25 °C for 5 minutes, and absorbance was measured at 560 nm. Ascorbic acid was used as a positive control. The scavenging activity was determined using the formula represented in the DPPH free radical scavenging assay section.

Reducing power

The reducing power was achieved according to the method reported by Bendjabeur et al.[22] In a 96-well microplate, 10 µl of sample solution at different concentrations was mixed with 40 µl of phosphate buffer (0.2 M, pH 6.6) and 50 µl of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 minutes, followed by adding 50 µl of trichloroacetic acid (10%), 40 µl of distilled water, and 10 µl of ferric chloride ($FeCl_3$, 0.1%). The microplate was vigorously shaken, and absorbance was immediately read at 700 nm. BHA, BHT, and ascorbic acid were used as antioxidant standards. The results were reported as absorbances, and the value of $A_{0.5}$ was calculated from the regression curve. $A_{0.5}$ corresponds to the concentration giving an absorbance of 0.5, which was determined from the regression curve.

Cupric reducing antioxidant capacity assay (CUPRAC)

CUPRAC was determined according to the method described by Apak et al.[23] The reaction medium included 40 µl of the sample at different concentrations, 50 mL of $CuCl_2$ (10 mM), 50 mL of neocuproine (7.5 mM in ethanol), and 60 mL of acetate ammonium (CH_3COONH_4, 1 M). After 1 hour of incubation, absorbance was measured at 450 nm. The results were reported as absorbance values and compared with BHA, BHT, and ascorbic acid.

β-carotene bleaching assay

The ability of the *P. venusta* extract to inhibit β-carotene bleaching was investigated according to the method of Ferhat et al.[24] with some modifications for this purpose, β carotene/linoleic emulsion was prepared by dissolving 0.5 g of carotenes in 1 mL of chloroform, 25 mL of linoleic acid, and 200 mg of tween 40. After removing the chloroform under vacuum using a rotary evaporator, the emulsion absorbance was adjusted to 0.8-0.9 at 470 nm by adding hydrogen peroxide. Then, 160 mL of the emulsion was mixed with 40 mL of the extract or standards. Finally, the microplate was incubated for 2 hours at 50 °C, and the absorbance was measured at 470 nm at different reaction times (t= 0 minutes and t=120 minutes). Ethanol was used as a control, and BHA and BHT were used as antioxidant standards. The results were given as IC_{50} using the following equation:

$$\textit{Inhibition (\%)} = \frac{As\ (t=0)\text{-}As\ (t=120)}{Ac\ (t=0)\text{-}Ac\ (t=120)} * 100$$

Where As (t= 0) and As (t= 120) are the absorbances of the sample at 0 and 120 minutes, respectively, Ac (t= 0) and Ac (t= 120) correspond to the absorbances of the control at 0 and 120 minutes.

Ferrous ions' chelating activities

The ferrous chelating activity was measured according to the method described by Decker and Welch.[25] Briefly, in a 96-well microplate, 40 µL of sample solution at different concentrations, 40 µl $FeCl_2$ (0, 2 mM), and 80 µl of ferene solution (0.5 mM) were mixed. After 10 minutes of reaction, the absorbance was measured at 593 nm. The metal chelating activity was calculated using the equation represented in the DPPH free radical scavenging assay section.

Statistical analysis

All measurements were performed in triplicate, and results were presented as means ± standard deviation. Student's t-test was used to determine the statistical significance of antioxidant activity using SPSS software. $p < 0.05$ was considered to be statistically significant.

RESULTS

Total polyphenol content

The results showed that the acetone extract of *P. venusta* contains a high level of total phenolic and flavonoid contents; the values were 123.42 ± 3.75 µg GAE/mg and 60.55 ± 0.81 µg QE/mg extract, respectively (Figure 3).

GC-MS analysis

The extract GC-MS profile revealed the presence of 13 different compounds, which were characterized and identified by comparison of their mass fragmentation patterns with those similar to those in the NIST library database. Among the identified compounds, Eugenol, Benzoic acid, 2, 4-dihydroxy-3, 6-dimethyl-, methyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z, Z)-, Tributyl acetyl citrate, Cyclopenta[a,d]cycloocten-5-one, 1,2,3,3a, 4,5,6,8,9,9a,10,10a-dodecahydro-7- (1-methylethyl)-1,9a-dimethyl-4-methylene, Hexanedioic acid, dioctyl ester, 2-Pentanoic acid, 5- (decahydro-5,5,8a-trimethyl-2-methylene-1-na phthalenyl)- 3-methyl-, [1S-[1α(E),4aβ,8aα]]-,3-Buten-2-one,3-methyl-4- (1,3,3-trimethyl-7-oxabicyclo [4.1.0] heptan-1-yl)-, 9,12-Octadecadienoic acid (Z, Z)-, octyl ester-, 9,10Anthracenedione, 1,8-dihydroxy-3-methoxy- 6-methyl- and Tetracosapentaene,2,6,10,15,19,23 hexamethyl- (Figure 4), (Table 1).

Quantification of usnic acid by HPLC

The content of usnic acid, one of the main compounds specific to lichens, in the extract was determined by HPLC. The results showed that the extract contained 0.0425 mg/mL of usnic acid (Figure 5), (Table 2).

Antibacterial activity

The antimicrobial effect of *P. venusta* acetone extract was assessed using disk diffusion and microdilution. The extract exhibited various antibacterial activities depending on the bacterial strains (Table 3). The largest inhibitory zone was recorded against *S. aureus* with 30 mm ± 0.01, followed by *E. coli* with 28 mm ± 0.01. The extract MIC was determined to be 6.25 mg/mL. However, usnic acid was only active on three bacterial strains, *E. coli, S. aureus,* and *B. subtilis*, at MIC 0.03 mg/mL, 0.03 mg/mL, and 0.015 mg/mL, respectively. However, no effect against *S. typhi* was observed by the lichen extract and usnic acid. By comparing the inhibition zone of usnic acid with that of the extract, it can be inferred that usnic acid is the most active component against both strains, *S. aureus and B. subtilis*. Notably, the extract and usnic acid have a more potent effect than the antibiotic gentamicin.

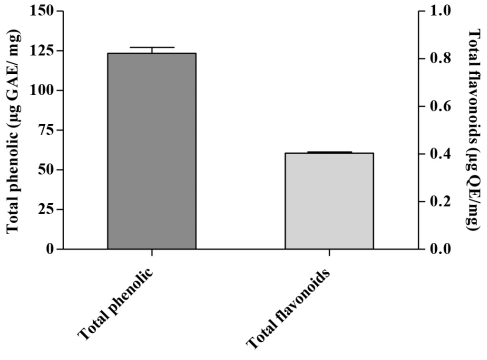

Figure 3. Total phenolic and flavonoids in the acetone extract of the lichen *Physconia venusta*

Antioxidant activity

The antioxidant potential of *P. venusta* extract was evaluated using different methods, and the results are presented in Table 4 as IC_{50} and $A_{0.5}$ values. The acetone extract of *P. venusta* exerted an interesting scavenging activity against the radical ABTS and the anion superoxide (IC_{50} = 20.00 ± 2.28 µg/mL and 24.80 ± 4.43 µg/mL, respectively). In contrast, it exhibited a weak effect against the DPPH radical. The extract also displayed a moderate ability to reduce ferric and cupric ions, incapacity to bleach β-carotene, and an excellent capability to bind ferreous ions (IC_{50} of 26.42 ± 2.98 µg/mL) compared with standard EDTA (IC_{50} = 12.11 ± 0.32 µg/mL).

DISCUSSION

Lichens are promising sources of bioactive molecules of pharmaceutical and nutritional interest. The emergence of antibiotic-resistant bacteria in healthcare is a serious concern. In the current research, the effect of the acetone extract of *P. venusta* on seven bacterial strains known for their resistance to antibiotics; *E. coli, P. aeruginosa, S. enteritidis, S. typhi, S. aureus, L. monocytogenes, and B. subtilis,* was investigated. The effect of the studied extract was compared with that of usnic acid, the essential metabolite found in lichens. The data showed that our extract has an antibacterial effect against the seven (7)

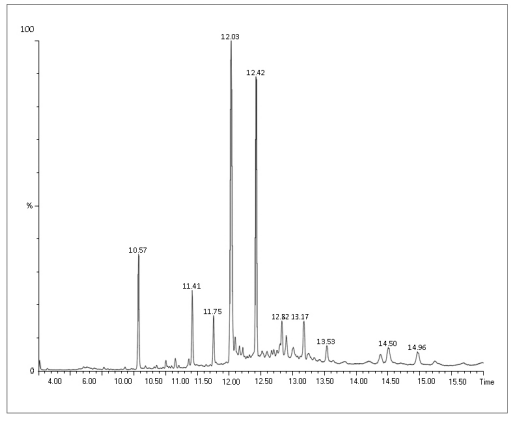

Figure 4. GC-MS chromatogram of *Physconia venusta* acetone extract
GC-MS: Gas chromatography-mass spectrometry

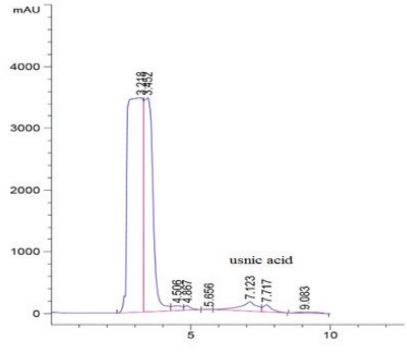

Figure 5. HPLC analysis of usnic acid in *Physconia venusta* acetone extract (retention time: 7,123 minute)
HPLC: High-performance liquid chromatography

strains at MIC 6.25 mg/mL, but the highest growth inhibition was observed against *S. aureus*.

Similarly, usnic acid showed an inhibition diameter close to that exhibited by the extract against the same bacterial strain, *S. aureus*, indicating that it is the principle active compound against this strain. The same observation was registered against *B. subtilis*, proving that usnic acid is the most active compound. Similar results were reported by Gupta et al.[26] with the acetone extract of the lichen *Bulbothrix setschwanensis*, which exhibited an antibacterial effect against *S. aureus* and *E. coli* at MIC 6.25 mg/mL. The study of Kosanić and Ranković[27] demonstrated that the acetone extract from *U. barbata* inhibited *S. aureus* at a MIC of 0.5 mg/mL and the usnic acid MIC was 0.125 mg/mL.[28] It also determined that extracts from the lichen *U. florida* showed an interesting antibacterial effect against methicillin-resistant and methicillin-sensitive strains of *S. aureus* with MICs of 100 and 850 µg/mL, respectively. Moreover, according to Londone Bailon et al.,[17] usnic acid extracted from *U. florida* has shown an antibacterial effect against the same bacteria with MICs of 100 and 750 µg/mL, respectively.

It has been reported that most of the antimicrobial molecules from lichens are polyphenols. Depsides, depsides, dibenzofurans, *etc*., isolated from lichens have also demonstrated significant antimicrobial effects. The suppression of topoisomerase, which is necessary for microbial replication, by polyphenols because of their affinity for binding to a variety of proteins, including enzymes, can also account for their antibacterial activity.[20]

On the other hand, the study of Pompilio et al.[29] showed that usnic acid induces cell wall damage and inhibits bacterial growth through the reduction of protein synthesis, which affects bacterial adhesion during the early stages of biofilm formation. It also reduces the pathogenic potential of *S. aureus* by affecting the expression of relevant virulence factors such as lipase and thermonuclease. The brine shrimp assay was performed to investigate whether the extract exerted more

Table 1. Identification of metabolites in the acetone extract of *Physconia venusta* by GC-MS analysis

	RT (minute)	Area %	Amount %	Compound detected	Molecule formula	Synonym
1	9.02	4.831	5.12	Eugenol	$C_{10}H_{12}O_2$	Eugenic acid
2	10.566	4.831	5.82	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	$C_{10}H_{12}O_4$	Atraric acid
3	11.412	3.547	8.44	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid
4	11.747	1.958	10.18	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid
5	12.027	19.232	10.50	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Alpha-linoleic acid
6	12.417	12.618	6.61	Tributyl acetyl citrate	$C_{20}H_{34}O_8$	Acetyl tributyl citrate
7	12.822	2.211	11.50	Cyclopenta[a,d]cycloocten-5-one,1,2,3,3a, 4,5,6,8,9,9a,10,10a-dodecahydro-7-(1-methylethyl)-1,9a-dimethyl-4-methylene	$C_{20}H_{30}O$	Adipic acid
8	12.892	1.107	5.75	Hexanedioic acid and dioctyl ester	$C_{22}H_{42}O_4$	Copalic acid methyl ester
9	13.172	2.146	11.16	2-Pentanoic acid, 5- (decahydro-5,5,8a-trimethyl- 2-methylene-1-na phthalenyl)-3-methyl-, [1S-[1α(E),4aβ,8aα]]-	$C_{21}H_{34}O_2$	-
10	13.533	1.259	6.55	3-Buten-2-one, 3-methyl-4- (1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)-	$C_{14}H_{22}O_2$	-
11	14.378	0.885	4.60	9,12-Octadecadienoic acid(Z,Z)-,octyl ester-	$C_{26}H_{48}O_2$	Physcion (parietin)
12	14.498	1.887	9.81	9,10Anthracenedione, 1,8-dihydroxy-3-methoxy-6-methyl-	$C_{16}H_{12}O_5$	-
13	14.958	1.516	7.88	Tetracosapentaene,2,6,10,15,19,23 hexamethyl-	$C_{30}H_{52}$	

GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

Table 2. Usnic acid content and retention time in the *Physconia venusta* acetone extract

	Usnic acid content (mg/mL)	% of usnic acid in dry weight	Retention time (minute)
P. venusta acetone extract	0.0425 ± 0.02	0.85 ± 0.01	7.123 ± 0.01

toxicity than an antibacterial effect; the results showed a SI > 1, indicating the extract's safety on living cells. In addition to their ability to be resistant to antibiotics, the seven selected strains can persist inside the host organism's cells and induce chronic inflammation.[30] This latter is characterized by excessive production of free radicals, leading to an imbalance between oxygen and nitrogen species (reactive oxygen species and reactive nitrogen species).[31] Thus, causing various tissue injuries like protein oxidation, lipid peroxidation, and DNA damage. Therefore, a dual-action antibacterial and antioxidant molecule would be of great therapeutic interest.[32,33]

The antioxidant activity of the extract was evaluated by different assays to consider the diverse mechanisms of action of bioactive compounds. The extract exhibited a potent free radical scavenging effect against ABTS and superoxide anions and an essential capacity for chelating ferrous ions. This ability to sequester transition metals is considered a valuable property of antioxidant compounds, which hinder the generation of free radicals such as OH• *via* the Fenton reaction, in which transition metals such as Fe^{2+} and Cu^{2+} play a significant role as catalysts. The action of antioxidants then form complexes characterized by a low redox potential while preventing them from participating in the reaction.[34]

The antioxidant activity of lichens has been the subject of different studies. Extracts from lichen species; *Cladonia furcata, Hypogymnia physodes, Lasallia pustulata, Parmelia*

Table 3. Antibacterial effects of *Physconia venusta* extract and usnic acid

Bacterial strains	Inhibition zone of the acetone extract (mm)[a]	Inhibition zone of usnic acid (mm)	% inhibition in liquid medium*	MIC of usnic acid (mg/mL)	MIC of the acetone extract (mg/mL)	SI	Gentamicin	Pristinamycin	DMSO
Escherichia coli (ATCC25922TM)	28 ± 0.01	-	50.90	0.03	6.25	2.8	2	15 ± 0.01	-
Pseudomonas aeruginosa (ATCC27853TM)	19 ± 0.01	-	34.54	-	6.25	2.8	1.7	-	-
Salmonella enteritidis (ATCC13076TM)	20 ± 0.02	-	36.36	-	6.25	2.8	-	-	-
Staphylococcus aureus (ATCC25923TM)	30 ± 0.01	27 ± 0.01	60	0.03	6.25	2.8	2.4	37 ± 0.01	-
Bacillus subtilis (ATCC6633TM)	10 ± 0.01	7 ± 0.02	18.18	0.015	6.25	2.8	-	17 ± 0.02	-
Listeria monocytogenes (ATCC 15313TM)	10 ± 0.01	-	18.18	-	6.25	2.8	-	-	-
Salmonella typhi (ATCC14028TM)	-	-	-	0.015	-	-	-	17 ± 0.01	-

Values are represented as mean ± SD of three measurements, a diameter of the disk (6 mm), (-) no inhibition, *values represent the inhibition percentages of *P. venusta* against different bacterial strains at 6.25 mg/mL using the microdilution method, ATCC: American Type Culture Collection, SI: Selectivity index, MIC: Minimum inhibitory concentration, DMSO: Dimethyl sulfoxide, SD: Standard deviation

Table 4. Antioxidant activity of the *Physconia venusta* acetone extract

Bacterial strains	DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)	O_2^- scavenging activity (IC_{50} µg/mL)	Reducing power ($A_{0.5}$ µg/mL)	CUPRAC ($A_{0.5}$ µg/mL)	β-carotene bleaching (IC_{50} µg/mL)	Fe^{2+} chelating activity (IC_{50} µg/mL)
P. venusta extract	> 800	20.00 ± 2.28[a]	24.80 ± 4.43[a]	162.67 ± 54.9[c]	164.78 ± 72.27[c]	NA	26.42 ± 2.98[a]
BHA	6.14 ± 0.41[a]	1.29 ± 0.30[a]	NT	9.29 ± 0.22[a]	5.35 ± 0.71[a]	0.91 ± 0.0[a]	NT
BHT	12.99 ± 0.41[b]	1.81 ± 0.10[a]	NT	8.41 ± 1.46[a]	8.97 ± 3.94[a]	1.05 ± 0.0[a]	NT
Ascorbic acid	4.39 ± 0.01[a]	3.04 ± 0.05[a]	7.59 ± 1.16[a]	3.62 ± 0.29[a]	8.31 ± 0.15[a]	NT	NT
EDTA	NT	NT	NT	NT	NT	NT	12.11 ± 0.32[b]

Values are mean ± SD of three measurements (n= 3). *BHA, BHT, ascorbic acid, and EDTA are the standards used. Values with different subscripts (a, b, c) in the same column are significantly different ($p < 0.05$),
DPPH: 2,2 diphenylpicrylhydrazyl, ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, SD: Standard deviation, NT: Not tested, EDTA: Ethylenediaminetetraacetic acid

caperata, Parmelia sulcata, Cetraria islandica, Usnea ghattensis, and *Usnea ghattensis* have shown an intense antioxidant activity.[35] Furthermore, compared with our data, the study of Ranković et al.[36] reported IC_{50} values relatively close to our results (0.78 to 6.25 mg/mL) for *C. furcata, H. physodes,* and *Umbilicaria polyphylla* extracts. However, extracts from *P. glauca* and *P. furfuraceae* lichens have a lower ability to reduce DPPH radicals, with IC_{50} values of 656.98 and 95.33 µg/mL, respectively.

The antioxidant properties of *P. venusta* can be attributed to its phenolic content or other secondary metabolites exclusively found in lichens. The antioxidant effect of polyphenols is broadly reported because of their structure bearing hydroxyl groups, and the number and location of these groups determine their antioxidant properties.[37] In the identical spectra, Fernández-Moriano et al.[44] showed a positive correlation between the antioxidant activity and the phenolic content of 10 *Parmeliaceae* lichen extracts*: Usnea contexta Motyka, Usnea aurantiacoatra, Parmelia omphalodes, Myelochroa irrugans, and Lethariella canariensis. Hypotrachyna cirrhata, F. haysomii, F. euplecta, F. caperata, and B. setschwanensis.*

Moreover, many components isolated from lichens exhibited strong scavenging activity. The antioxidant activity of usnic acid was further assessed *in vivo* and *in vitro*. It was reported to reduce oxidative damage by increasing glutathione peroxidase, constitutive nitric oxide synthase, and superoxide dismutase activities *in vivo*.[38] It is a potent scavenger of peroxyl radicals assessed by ORAC.[38] Other compounds like depsides, artistic acid, 8'-methylmenegazziaic, psoromica, and protocetraric acid also have free radical scavenging activity.[39] Lichens produce many of the fatty acids commonly found in higher plants; the major fatty acid compositions are oleic, linoleic, and palmitic acid.[40] Molina et al. [41] studied the lichen *Physconia distorta* and suggested a close relationship between the synthesis of secondary metabolites and fatty acid metabolism. Mycobiota grown in a glucose-enriched medium favored the production of fatty acids. The antibacterial activity of fatty acids such as linoleic acid, palmitoleic acid, oleic acid, and their esters is well-known against Gram-negative and Gram-positive bacteria. Linoleic acid, in addition to its anticancer properties,[42] has been reported to inhibit the growth of *S. aureus* by increasing its permeability.[43] According to Table 1, we could identify several chemicals, most of which have biofunctions; however, as the main limitation of our study, we did not directly and individually assess their properties. In addition, we recommend using further bacteria to have a more trusted view and insight into the extract and its components on antimicrobial properties.

CONCLUSION

In the current investigation, *P. venusta* extract was shown to possess antibacterial activity against several tested bacterial strains that were resistant to antibiotics and caused nosocomial and chronic illnesses. This study also demonstrated that usnic acid, the most researched lichen component, had potent antibacterial properties against *B. subtilis* and *B. aureus*. The *P. venusta* extract also has an essential ability to scavenge free radicals. Because of its potent properties, *P. venusta* is an excellent natural source for the development and discovery of novel compounds with significant pharmaceutical potential.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Authorship Contributions

Surgical and Medical Practices: I.Z., M.A.T., M.B.M., Concept: I.Z., I.E., C.B., Design: I.Z., I.E., C.B., Data Collection or Processing: I.Z., Analysis or Interpretation: I.Z., M.B.M., D.E-A-K., L.G., Literature Search: I.Z., M.A.T., M.B.M., D.E-A-K., Writing: I.Z., D.E-A-K.

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