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GC-MS profiling and pharmacological potential of *Physconia venusta (Ach.) Poelt*

Short Title in English: Pharmacological potential of *Physconia venusta*

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Abstract
Objectives: Lichens are complex symbiotic organisms that generate various bioactive compounds with significant therapeutic value. We sought to investigate the chemical composition and bioactivity of the acetone extract of the Algerian lichen *Physconia venusta (Ach.) Poelt*.
Materials and Methods: Phytochemical screening was performed using GC-MS. The antibacterial activity was assessed against *Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhi, Staphylococcus aureus, listeria monocytogenes,* and *Bacillus subtilis* using 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). Additionally, cytotoxic activity was tested using *Artemia salina* (Brine shrimp) bioassay.
Results: The studied extract exhibits intense antibacterial activity against *E. coli* and *S. aureus* with inhibition diameters of 28±0.01 and 22±0.01 mm respectively, with the 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 the presence of 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 made up 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 having a wide range of activities, including anti-inflammatory, anti-proliferative, cytotoxic, and anticancer properties, have been described by previous research. [2] More than 1085 lichen species have been identified in Algeria, 64 of which are indigenous. [3]

Their usage as bioindicators of air pollution has received the majority of scientific attention. However, there are very few scientific studies on lichen chemistry in this highly diverse country. Antibiotic resistance in bacteria is one of the major problems 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 being 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, TB, sepsis, and foodborne illnesses, as a result of antibiotics losing their potency. The estimate is that by 2050, an effective antibiotic will only be available if new drugs are developed or discovered. [5] Because of this, 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 the secondary lichen metabolite. Numerous researches 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 their levels and the usnic acid content.

One of the rare lichens with a distribution centered in Italy, Morocco, and Algeria is *Physconia venusta (Ach.) Poelt,* 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 responsible for 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 1a) was collected from Elmeridje forest-Constantine in the Northeast of 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 stored in the dark until extraction.

Preparation of acetone extract from *Physconia venusta*

Acetone has been effectively used to extract antioxidants including phenolics due to 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 20g of powder in 100 ml of acetone at room temperature for 72h. Following filtering, the mixture was concentrated in a rotary evaporator (Buchi 23022A120 Rotavapor Cole-Parmer, USA). The obtained extract was finally stored at 20°C.

Chemical characterization

Total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent according to the method described by Müller et al [12]. Briefly, 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, replacing the extract with the used solvent.

Total flavonoid content

Total flavonoid content was measured according to the method of Topçu et al [13] with some modifications for a 96-well microplate assay. Briefly, 50µl of the extract (1mg/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 min, and the absorbance was measured at 415 nm.

GC-MS Analysis

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

Quantification of usnic acid by HPLC

high-performance liquid chromatography (HPLC) analysis was performed according to 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 with seven samples of various concentrations using linear regression analysis (Figure 1b)

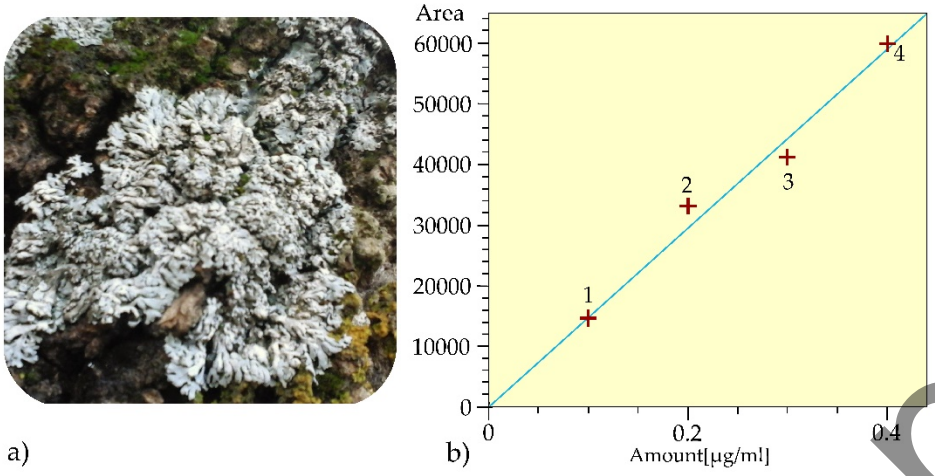

Figure 1a) Algerian *Physconia venusta* photographed in the Elmeridje forest-Constantine, Algeria, b) Calibration curve of usnic acid (Sigma);R_2=0.9896.

The analysis was performed on Agilent 1220 infinity LC system equipped with a diode array detector; a reverse phase C18 column was used. The 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. 20µl of the filtered solution was injected into the HPLC system. All experiments were performed in triplicates.

Antimicrobial activity

Microorganisms and media

The Constantine Research Center in Biotechnology- Algeria provided the bacterial strains including four Gram-negative: *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 18h 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 (15µl/disk) at two different concentrations (50 mg/ml and 1mg/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, while gentamicin was used as a positive control. The inhibition zones were reflective of the antimicrobial effectiveness of the extract.

Minimal inhibition concentration

The minimal inhibitory concentration (MIC) was determined by the broth microdilution method using 96-well micro-titer plates according to the method described by Londone and colleagues. [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 respectively used in the experiment against every tested strain. 50µl of a bacterial inoculum (107CFU/ml) was transferred in each well containing 50 µl of lichen extract or usnic acid solution. After 24h of incubation at 37°C, the absorbance was measured at 630 nm, and the minimal inhibitory concentration was determined. This later corresponded to the lowest concentration, which completely inhibited bacterial growth. A DMSO solution was used as a negative control, and a culture medium with bacteria was used as a 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 (10g /1L of seawater). The solution was incubated at 28°C for 48h 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 the Brine shrimp larvae viability was assessed using the

method reported by Sahely Sarah et al. [18] 10 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 h of incubation, the number of surviving larvae in each tube was determined. Potassium dichromate was used as a positive control. The percentage of mortality was calculated employing 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

antimicrobial selectivity index (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:

Selectivity index = LC_{50} (mg/ml)/MIC (mg/ml)

Where LC_{50} refers to the concentration of the sample inducing 50 % lethality of the *artemia nauplii*, MIC represents the minimum inhibitory concentration.

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 min. 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 DPPH radical was calculated using the following equation:

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

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

ABTS assay

The ABTS scavenging activity was evaluated according to the method described by Londone et al. [17] The ABTS solution was prepared by mixing seven mM of ABTS with 2.45 mM of potassium per sulfate for 16h. The $ABTS^{+\bullet}$ solution was then diluted in distilled water to an absorbance of 0.708±0.025 at 734 nm. In 96 wells microplate, 40 µL of the sample at different concentrations were mixed with 160 µL of the $ABTS^{+\bullet}$ solution. After 10 min of incubation, the 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 DPPH free radical scavenging assay section.

Superoxide anion scavenging activity

The superoxide radical scavenging ability was assessed by measuring the inhibition of O2− 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 (NBT) solution (1 mg/mL in DMSO). The mixture was incubated at 25°C for 5 min, and the absorbance was measured at 560 nm. Ascorbic acid was used as a positive control. The scavenging activity was determined using the formula represented in 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 min, 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 the absorbance was immediately read at 700 nm. BHA, BHT, and ascorbic were used as antioxidant standards. The results were reported as absorbances, and the value of $A_{0.5}$ was calculated from the regression curve. $A_{0.5}$ correspond to the concentration giving an absorbance of 0.5 was determined from the regression curve.

Cupric reducing antioxidant capacity assay (CUPRAC)

The cupric-reducing antioxidant capacity (CUPRAC) was performed 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 one hour of incubation, the absorbance was measured at 450 nm. The results were reported as absorbance values and were compared to 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 gr carotenes in 1 ml of chloroform, 25 ml of linoleic acid, and 200 mg of Tween 40. After removing the chloroform under a 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 extract or standards. Finally, the microplate was incubated for two hours at 50°C, and the absorbance was measured at 470 nm at different reaction times (t=0 min and t=120 min). Ethanol was used as a control, whereas BHA and BHT were used as antioxidant standards. The results were given as IC_{50} using the following equation:

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

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

Ferrous ions' chelating activity

the ferrous chelating activity was measured according to the method described by Decker et al. [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 min of reaction, the absorbance was measured at 593 nm. The metal chelating activity was calculated using the equation represented in DPPH free radical scavenging assay section.

Statistical analysis

All measurements were performed in triplicates, and results were presented as means ± standard deviation (SD). 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 polyphenols 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 2a)

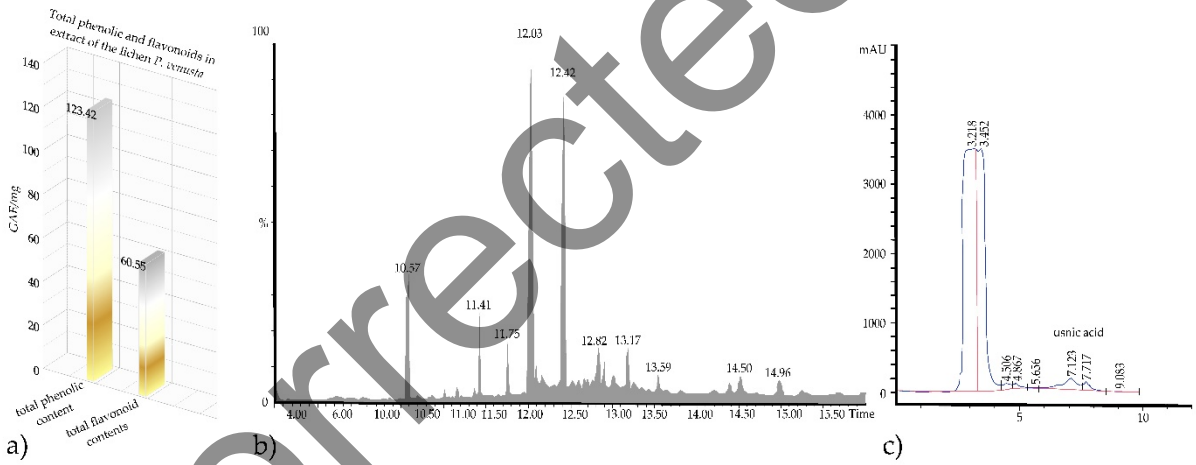

Figure 2a) Total phenolic and flavonoids in the acetone extract of the lichen *P. venusta, b)* GC-MS chromatogram of *P. venusta* acetone extract, c) HPLC analysis of usnic acid in *P. venusta* acetone extract (Retention time: 7,123 min).

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 pattern with those similar 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- (Table.1)(Figure2b)

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

	RT(min)	Area %	Amount %	Compound detected	Mol. 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, 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 hexamet hyl-	$C_{30}H_{52}$	

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 contains 0.0425 mg/ml of usnic acid. (Table.2)(Figure 2c)

Table 2. Usnic acid content and retention time in P. venusta acetone extract

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

Antibacterial activity

The antimicrobial effect of *P. venusta* acetone extract was assessed using disc diffusion and microdilution methods. 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 at 6.25 mg/ml. However, the 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 the usnic acid**.** By comparing the inhibition zone of the 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 the usnic acid have a more potent effect than the antibiotic gentamicin.

Table 3. Antibacterial effect of *P. venusta* extract and usnic acid

bacterial strains	Inhibition zone of acetone extract (mm)[a]	Inhibition zone of usnic acid (mm)	% of inhibition in liquid medium *	MIC of usnic acid (mg/ml)	MIC of acetone extract (mg/ml)	Selectivity index (SI)	gentamicin	Pristinamycin	DMSO
E. coli (ATCC25922TM)	28 ± 0.01	-	50.90	0.03	6.25	2.8	2	15±0.01	-
P. aeruginosa (ATCC27853TM)	19 ± 0.01	-	34.54	-	6.25	2.8	1.7	-	-
S. enteritidis (ATCC13076TM)	20 ± 0.02	-	36.36	-	6.25	2.8	-	-	-
S. aureus (ATCC25923TM)	30 ± 0.01	27±0.01	60	0.03	6.25	2.8	2.4	37±0.01	-
B. subtilis (ATCC6633TM)	10 ± 0.01	7±0.02	18.18	0.015	6.25	2.8	-	17±0.02	-
L. monocytogenes (ATCC 15313TM)	10 ± 0.01	-	18.18	-	6.25	2.8	-	-	-
S. typhi(ATCC14028TM)	-	-	-	0.015	-	-	-	17±0.01	-

Values were represented as means ± SD of three measurements, a diameter of the disc (6 mm). (-) no inhibition.
* The values represent the inhibition percentages of *P. venusta* against the different bacterial strains at 6.25 mg/ml using the microdilution method

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. As shown, 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 has also displayed a moderate ability to reduce ferric and cupric ions, incapacity to bleaching β-carotene, and an excellent capability to bind ferreous ions (IC_{50} of 26.42±2.98 µg/ml) compared to the standard EDTA (IC_{50}= 12.11±0.32 µg/ml).

Table 4. Antioxidant activity of *P. 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 means ± SD of three measurements (n=3). * BHA, BHT, ascorbic acid, and EDTA are the applied standards. Values with different subscripts (a, b, c) in the same column are significantly different ($p<0.05$), NT: Not tested.

Discussion

Lichens are a promising source 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 to the effect of the usnic acid, the essential metabolite found in lichens. The data showed that our extract has an antibacterial effect against the seven (7) strains at MIC 6.25 mg/ mL, but the highest growth inhibition was observed against *S. aureus*.
Similarly, the usnic acid has shown 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* that exhibited an antibacterial effect against *S. aureus* and *E. coli* at MIC 6.25 mg/mL. The study of Kosanić et al [27] demonstrated that the acetone extract from *U. barbata* inhibited *S. aureus* at 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 et al. the usnic acid extracted from *U. florida* has shown an antibacterial effect against the same bacteria with MIC of 100 and 750 µg/mL, respectively.
It was reported that most of the antimicrobial molecules from lichens are polyphenols**.** Depsides, depsidones, dibenzofurans, etc., isolated from lichens have also demonstrated significant antimicrobial effects. The suppression of topoisomerases, which are necessary for microbial replication, by polyphenols due to 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. Indeed, the brine shrimp assay was performed to investigate whether the extract had exerted more toxicity than an antibacterial effect; the results showed a selectivity index >1, indicating the extract's safety on the living cells. In addition to their ability to be resistant to antibiotics, the seven chosen 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 (ROS and RNS). [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]
On the other side, the antioxidant activity of the extract was evaluated by different assays to take into account the diverse mechanisms of action of bioactive compounds. The extract exhibited a potent free radical scavenging effect against ABTS and superoxide anion and an essential capacity for chelating ferrous ions. This ability to sequester transition metals is considered a valued 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 forms complexes characterized by a low redox potential while preventing them from participating in the reaction. [34]
The antioxidant activity of lichens was the subject of different studies. Extracts from lichen species; *Cladonia furcata, Hypogymnia physodes, Lasallia pustulata, Parmelia caperata, Parmelia sulcata, Cetraria islandica, Usnea ghattensis, and Usnea ghattensis* have shown an intense antioxidant activity. [35] Furthermore, compared to our data, the study of Rankovi et al [36] reported IC50 values relatively close to our results (0.78 to 6.25 mg/mL) for *Cladonia furcata, Hypogymnia physodes,* and *Umbilicaria polyphylla* extracts. However, extracts from *P. glauca* and *P. furfuraceae* lichens have a lower ability to reduce DPPH radical with IC_{50} values of 656.98 and 95.33µg/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 due to their structure bearing hydroxyl groups, and the number and the location of these groups determine their antioxidant properties. [37] In the identical spectra, Moriano et al showed a positive correlation between the antioxidant activity and the phenolic content of ten *Parmeliaceae* lichen extracts*: Usnea contexta Motyka, Usnea aurantiacoatra, Parmelia omphalodes, yelochroa irrugans, Lethariella canariensis. Hypotrachyna cirrhata, Flavoparmelia haysomii, Flavoparmelia euplecta, Flavoparmelia caperata and Bulbothrix setschwanensis.*
Moreover, many components isolated from lichens exhibited a strong scavenging activity. The antioxidant activity of usnic acid was furthermore 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 depsidones, norstistic acid, 8'-methylmenegazziaic, psoromic, 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 acids compositions are oleic, linoleic, and palmitic acid. [40] Molina et al. 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. [41] 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 anti cancer properties [42], has been reported to inhibit the growth of S. aureus through increasing the 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. Additionally, 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 a wide range of tested bacterial strains that were resistant to antibiotics and the cause of 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 prospects.

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