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ORIGINAL PAPER

In vitro evaluation of lysozyme activity and antimicrobial effect of extracts from four Tunisian lichens: Diploschistes ocellatus, *Flavoparmelia caperata, Squamarina cartilaginea and Xanthoria parietina*

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Abstract
Since lichens have been recognised as a potential natural source of bioactive substances, the aim of this study was to investigate the antimicrobial, lysozyme and antifungal effects of methanol, acetone and quencher extracts from four lichens: *Diploschistes ocellatus*, *Flavoparmelia caperata*, *Squamarina cartilaginea* and *Xanthoria parietina.* The results showed that the tested extracts had antimicrobial activity against Gram-positive and Gram-negative bacteria and anti-candida, and inhibit the spore germination of tested fungi. The different extracts varied in their effect as determined by the diameter of the inhibition zone, the highest values being observed with the methanol and acetone extracts (29.5 and 27.5 mm, respectively) for *S. cartilaginea* against *Enterococcus faecalis*. For powdered material (quencher), *F. caperata* showed the highest inhibition diameter (25.5 mm) against *Staphylococcus aureus.* The Minimum Inhibitory Concentration (MIC) values varied from 125 to 2000 µg mL^{-1}. Methanol extracts of *S. cartilaginea* were more active against *Enterobacter cloacae* (MIC 125 µg mL^{-1}) and *Staphylococcus aureus* (MIC 125 µg mL^{-1}), and also affected lysozyme activity against *Staphylococcus aureus*, as well as the morphology of fungal hyphae. This study demonstrated that the investigated species are a potential source of bioactive compounds which are potentially important antimicrobial agents.

Keywords Antifungal potential · Antimicrobial power · Lichenicolous fungi · Lysozyme effect · Spore germination

Introduction

For centuries, humans have used plants to treat common infectious diseases, and sometimes lichens have been employed for this purpose. Lichens are symbiotic organisms essentially composed of two distinct individuals: a fungus (heterotrophic) and an alga or cyanobacterium (autotrophic). Lichens provide natural resources (Kosanić et al. 2012) and produce a wide variety of secondary metabolites, many of which are specific to lichens (Thadhani et al. 2011). Huneck and Yoshimura (1996) listed more than 1000 lichen metabolites and Stocker-Wörgötter (2008) reported that many of these are known to have multiple pharmacological activities such as antibiotic, antiviral, anti-inflammatory, antioxidant and cytotoxic effects; in fact, 50% of all lichens have antibiotic capabilities (Ranković 2015).

Lichen-derived products have antibiotic properties of particular interest to scientists. A large number of reports concerning the antimicrobial properties of lichens have appeared in the literature (e.g. Nugraha et al. 2020, Soundararajan et al. 2019, and Tas et al. 2019). Kosanić et al. (2012) discovered an antimicrobial potential for the lichens *Umbilicaria crustulosa*, *U. cylindrica* and *U. polyphylla*. This was also demonstrated by Ranković (2015) who noted that 52 genera of lichens are used in traditional medicines because

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of their beneficial effects on human health. The beneficial effect of lichens is due to the richness of secondary compounds. Ganesan et al. (2015) showed that the presence of phenolic compounds, such as flavonoids, phenolic acids and total phenols, will determine the biological activity of lichens. Moreover, Mendili et al. (2019) have shown a higher phenolic content in *Diploschistes ocellatus* and important antioxidant activity; this species also showed an antibacterial effect (Almola et al. 2016). *Flavoparmelia caperata* and *Xanthoria parietina* are also known for their richness in lichen compounds and for their biological activity (Dieu et al. 2019; Basile et al. 2015; Dias and Urban 2009; Gupta et al. 2007). Gomez-Serranillos et al. (2014) demonstrated that the family of *Parmeliaceae* has an important antimicrobial activity. To date, very little research has been published on the chemical components of *Squamarina*, but it is known that squamarone and psoromic acids, present in this genus, have powerful antibacterial activity (Zheng et al. 2019).

The present study shows that some Tunisian lichens are a very interesting and potentially important source of bioactive compounds, particularly antimicrobial agents. Therefore, the present work investigated in vitro the antimicrobial potentialities of extracts from four lichens, *Diploschistes ocellatus* (Vill.) Norman, *Flavoparmelia caperata* (L.) Hale, *Squamarina cartilaginea* (With.) James, and *Xanthoria parietina* (L.) Beltr., collected from Tunisia.

Materials and methods

Sample preparation and extraction

Samples of *D. ocellatus*, *S. cartilaginea* and *X. parietina* were collected from the Bazina region of Tunisia (36°96′05.80″ N, 09°29′ 73.84″ E) and *F. caperata* from the Nefza region of Tunisia (37°02′11″ N, 9°05′12″ E) in February 2016. Voucher specimens have been deposited in the Lichenological Herbarium of the Department of Biology, Faculty of Sciences, University of Tunis El-Manar. Extracts from finely fragmented dry lichens thalli were derived by means of acetone and methanol (40 g of thalli per 200 mL for each solvent). The ultrasonic extraction was conducted for 2 h at room temperature. The extracts were filtered and then concentrated in a rotary evaporator. The crude extracts were kept at + 4 °C until analysed.

Antimicrobial assessment of powdered material by the QUENCHER approach

The QUENCHER approach (Quick, Easy, New, Cheap, Reproducible) is a method for the quantification of phenolic compounds and the measurement of total antioxidant power of foods (Gökmen et al. 2009). This method was used to measure the antimicrobial capacity of the powdered of the four lichens. After dilution with an inert material, in this case microcrystalline cellulose, the samples were analysed in the same way.

Antimicrobial assays

Microorganisms

To determine the antimicrobial potential of the lichens extracts, Gram-negative bacteria (*Enterobacter cloacae* and *Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*), yeasts (*Candida albicans*, *Candida sake* and *Candida parapsilosis*) and fungi (*Penicillium* spp., *Aspergillus* spp., *Alternaria alternata* and *Colletotrichum acutatum*) were obtained from the Culture Collection of the Laboratory of Mycology, Pathology and Biomarkers, Faculty of Sciences in Tunisia. The pathogenic strains used in this work were obtained from human clinical samples of Tunisians in isolation. The identification was based in phenotypic and genotypic criteria. The cultures medium was prepared in Tryptone Soy Broth (TSB; 50 mL) (Bio-Rad, France), and held in a 100-mL Erlenmeyer flask inside an orbital incubator (110 rpm, 37 °C). TSB (25 mL) was used to prepare bacterial cultures for the suspension tests. TSB and TSA were employed for all experiments involving bacteria; for *Candida* spp., yeast malt extracts broth (YMB: Bio-Rad, France) and agar (YMA: Bio-Rad, France) were used, and for fungi, potato dextrose agar (PDA) was used. TSB, TSA and PDA media were dissolved in distilled water, brought to the boil with frequent shaking to ensure they were completely dissolved, and sterilised by autoclaving at 121 °C for 15 min.

Agar diffusion method

Agar media were autoclaved, cooled to 45 °C, and seeded at 1% (v/v) with a culture of the overnight indicator strain. After homogenizing, the 25 mL of agar was poured into Petri dishes (90 mm diam.), which were left at 25 °C for solidification. Before use, each lichen extract was diluted in distilled water, adjusted to 2000 µg mL^{-1}, and sterilised by filtration through a 0.22-µm pore size filter. A 50-µL aliquot of the filtered extracts was placed into paper discs. After overnight pre-diffusion at 4 °C, the plates were incubated at temperatures of 37 °C or 30 °C, respectively for bacteria and fungi for at least 24 h to develop inhibition zones, the diameters of which were measured in mm.

Determination of minimum inhibitory concentration (MIC)

The MIC (μg mL^{-1}), denoting the lowest inhibition concentration of the evaluated antimicrobial product that prevents the visible growth of tested microorganisms, was evaluated by the serial double dilution method in an appropriate medium inoculated with a standardised number of microorganisms. The concentration of each extract incubated with the indicator strain is given in μg mL^{-1}. Each extract diluted in 1000 μL of sterile distilled water was inoculated with 100 μL of 10^6 UFC mL^{-1} of each indicator strain and the different culture tubes were incubated at the appropriate temperature (see above). Control tubes containing 100 μL of 10^6 UFC mL^{-1} of each indicator strain were added to 1000 μL of culture medium lacking lichen extracts. MIC was estimated visually (absence of turbidity) and determined with three independent measurements (Jorgensen and Turnidge 2007).

Resolution of bactericide activity

The antimicrobial potential of each of the four lichens extracts was expressed in arbitrary units per ml (AU mL^{-1}) and determined by an agar diffusion assay as described by Graciela et al. (1995). Briefly, a serial twofold dilution in sterile distilled water of the lichens extracts was prepared, and 50 μL of each dilution was spotted onto a TSB agar soft plate seeded with 10^5 CFU mL^{-1} of *S. aureus*. The AU (arbitrary unit) mL^{-1} was calculated as: $AU\ mL^{-1} = 1000 \times D/A$ (where: *A* is the volume of the lichens extract, aliquot spotted on agar plate (50 μL in this case); *D* is the reciprocal of the highest dilution showing a clear inhibition of the indicator strain).

Lysozyme activity

The Lysozyme activity of each lichen extracts was assayed turbidimetrically by measuring the decrease in absorbance at 660 nm of a suspension of *S. aureus* and *S. agalactiae* (Ryazanova et al. 2005). After culturing *S. aureus* and *S. agalactiae* for 48 h at 30 °C, the bacterial cell was centrifuged, washed twice with distilled water, and suspended in 50 mM sodium phosphate buffer at pH 6.5. Subsequently, the bacterial suspension volume by volume (v/v) and the lichen extracts were incubated at 37 °C for 30 min. One unit of lysozyme activity was determined as the decrease in OD at 660 nm with 0.01 per min, compared to the control tube containing the volume (v) of pathogenic bacteria and the volume (v) of buffer without the extracts (Essghaier et al. 2014).

Antifungal activity

Spore germination inhibition

All tested fungi were grown at 25 °C on PDA for 10–15 days. Sterile water (20 mL) was added to each plate, and the surface was scraped gently with a sterile loop to release the spores. The resulting spore suspension was filtered through a sterile 30-μm filter to remove any mycelial fragments. The conidial suspension of each fungus was adjusted to 10^4 spores per mL by means of a haemocytometer. To investigate the effects of various extracts from the four lichens on spore germination, 20 μL of conidial suspension (10^4 spores per mL^{-1}) and 20 μL of each extract (5000 μg mL^{-1}) were pipetted into an Eppendorf tube containing 1 mL of sterile distilled water with 5% glucose; the mix was then incubated at 21 °C for 24 h. Control tubes were inoculated with fungal spores of each tested fungus. The percentage of spore germination inhibition (*I*%) was determined by microscopic examination for each extract (*E*) and compared to the control tube containing only the spore suspensions from the formula: $I\,(\%) = (C - E)/C \times 100$ (where: *C* denotes the number of spores counted in the control tube and *E* the number of spores in the tube containing the suspension of spores and the extracts). Three replicates were used for each treatment (Sarangi et al. 2010).

Mycelial hyphae destruction

Each fungal culture was rinsed with distilled sterile water; after centrifugation at 9000 rpm for 10 min, the pellet (mycelium) was replaced in an Eppendorf tube containing a Tris–HCl buffer (0.01 M, pH 8) to obtain the same concentration of mycelial solution (expressed in mg mL^{-1}). 500 μL of the methanol extracts (5000 μg mL^{-1}) was added, and the mixture incubated at 37 °C for 14 h. Three replicates were used for each test. Optical density (OD) was measured at 540 nm. The increase of OD was compared with the control tube (containing only a mycelial suspension) to confirm the destruction of fungal hyphae by the extracts. The ratio between the OD of the extract and OD of the control as a function of the volume used 2 mL (Extract + mycelium with Tris–HCl buffer) and as a function of incubation time 14 h were determined (Ryazanova et al. 2005).

Results

This study demonstrates the antimicrobial activities of *D. ocellatus*, *F. caperata*, *S. cartilaginea* and *X. parietina* extracts (methanol, acetone and quencher) against bacteria, yeasts and fungi. Noticeable antibacterial activities were revealed by these four lichens with different extracts

Table 1 Antibacterial activity detection of the tested lichen species expressed as diameter zone of the inhibition (in mm)

Lichen species	Microorganisms							
	Extract	*Staphylococcus aureus*	*Enterobacter cloacae*	*Streptococcus agalactiae*	*Escherichia coli*	*Candida albicans*	*Candida parapsilosis*	*Candida sake*
Diploschistes ocellatus	Methanol	19.5 ± 0.7	15 ± 0	_	13.25 ± 0.5	_	12.5 ± 0.4	12 ± 0
	Acetone	13.75 ± 0	16.25 ± 0.2	_	11.5 ± 0.1	_	13.5 ± 05	_
	Quencher	17.75 ± 0.3	_	15.25 ± 0.3	_	_	_	16.5 ± 0.3
Flavoparmelia caperata	Methanol	24 ± 0	20 ± 0.34	13.5 ± 0.5	23 ± 0	_	_	13.5 ± 0.2
	Acetone	16 ± 0	24.5 ± 0.5	_	17.5 ± 0.5	_	15 ± 0	14.5 ± 0.35
	Quencher	25.5 ± 0.14	23 ± 0	17.5 ± 0.2	22.5 ± 0.1	14.5 ± 0.25	_	21 ± 0
Squamarina cartilaginea	Methanol	21.25 ± 0.2	29.5 ± 0.05	14.5 ± 0.35	23.5 ± 0.5	14.5 ± 0.1	_	14.5 ± 0.25
	Acetone	17 ± 0.1	27.5 ± 0.3	_	21.5 ± 0.25	15.5 ± 0.3	17.25 ± 0.25	14 ± 0
	Quencher	15.75 ± 0.17	20.5 ± 0.1	_	22.25 ± 0.3	12 ± 0	_	19.5 ± 0.5
Xanthoria parietina	Methanol	16 ± 0.5	14.25 ± 0.25	_	_	_	_	10.5 ± 0.1
	Acetone	23. 5 ± 0	_	11 ± 0	_	12.5 ± 0.5	_	10.5 ± 0.25
	Quencher	16.25 ± 0.14	12.5 ± 0.2	_	_	_	_	15.5 ± 0.5

(Table 1). The results show a large variability of values ranging from 29.5 to 11 mm; the highest inhibition diameter (29.5 mm) was observed with the methanol extract of *S. cartilaginea* against *E. cloacae*, followed by the acetone extract of the same lichen (27.5 mm). A diameter inhibition of 23.5 mm was obtained against *S. aureus* and *E. coli* with the acetone extract from saxicolous *X. parietina* and the methanol extract from *S. cartilaginea*; the extraction solvent appeared to have no effect on the diameter zone of inhibition. However, *S. cartilaginea* is very active and has a very strong antimicrobial power irrespective of the extraction solvents acetone and methanol.

For the quencher extracts, *F. caperata* showed the high inhibition diameter (25.5 mm) against *S. aureus*, and 23 mm against *E. cloacae*, but the inhibition diameter of *S. agalactiae* was only 17.5 mm by this method.

The results of anti-yeast activity obtained showed notable differences between lichen species and the extracts tested (Table 1). The quencher extracts of *F. caperata* and *S. cartilaginea* exhibited high inhibition diameters (21 mm and 19.5 mm, respectively) against *C. sake*.

The MIC (Minimum Inhibitory Concentration) values given in Table 2 show the differences between the various extracts used. Quencher extracts were only active for

Table 2 The minimal inhibition concentration (MIC in µg/mL) of the methanol, acetone and quencher extracts of *Diploschistes ocellatus*, *Flavoparmelia caperata*, *Squamarina cartilaginea* and *Xanthoria parietina* against the microorganisms tested using microdilution assay

Lichen species	MIC in µg mL^{-1}							
	Extract	*Staphylococcus aureus*	*Enterobacter cloacae*	*Streptococcus agalactiae*	*Escherichia coli*	*Candida albicans*	*Candida parapsilosis*	*Candida sake*
Diploschistes ocellatus	Methanol	2000 ± 1.5	250 ± 0	_	250 ± 0.2	_	2000 ± 0	2000 ± 0.6
	Acetone	250 ± 0.5	2000 ± 1.4	_	125 ± 0	_	2000 ± 0	_
	Quencher	2000 ± 0.9	_	2000 ± 0	_	_	_	2000 ± 1.06
Flavoparmelia caperata	Methanol	2000 ± 0.7	250 ± 0	2000 ± 0.4	250 ± 0.5	_	_	2000 ± 0.5
	Acetone	250 ± 0.7	2000 ± 0.5		125 ± 0.2	_	250 ± 0.1	2000 ± 0.35
	Quencher	2000 ± 1.6	2000 ± 1	2000 ± 0.9	2000 ± 1.5	2000 ± 0.8	_	2000 ± 0.7
Squamarina cartilaginea	Methanol	125 ± 0.5	125 ± 0	2000 ± 0	2000 ± 07	250 ± 0	_	250 ± 0
	Acetone	2000 ± 1.2	250 ± 0.2	_	2000 ± 0.3	500 ± 0.5	500 ± 0.5	2000 ± 1.5
	Quencher	2000 ± 1	500 ± 0.5	_	2000 ± 0.1	2000 ± 0.7	_	250 ± 0.2
Xanthoria parietina	Methanol	2000 ± 0.6	2000 ± 0.7	_	_	_	_	2000 ± 0
	Acetone	250 ± 0.3	_	2000 ± 0.2	_	500 ± 0	_	2000 ± 0.7
	Quencher	2000 ± 0	2000 ± 0.5	_	_	_	_	500 ± 0.2

S. cartilaginea against *E. cloacae* at 500 µg mL^{-1}, but inactive for the other species against bacterial species. However, methanol extracts of *S. cartilaginea* were more active against *E. cloacae* and *S. aureus* at 125 µg mL^{-1}, and acetone extracts of *D. ocellatus* and *F. caperata* were more effective against *E. coli* at only 125 µg mL^{-1}.

The MIC value of the lichen species extracted varied from 2000 to 250 µg mL^{-1}. The methanol extract of *S. cartilaginea* was the most active against *C. albicans* and *C. sake* with 250 µg mL^{-1}. The acetone extract of *F. caperata* was able to inhibit *C. parapsilosis* with 250 µg mL^{-1}, and *S. cartilaginea* inhibited *C. parapsilosis* with 500 µg mL^{-1}.

The quencher extracts were able to inhibit all microorganisms tested in this study at the same MIC value which varied from 2000 to 250 µg mL^{-1}, but they failed to inhibit *C. parapsilosis* (Table 2). Differences in the results related to microorganisms can be explained by the individual sensitivity of tested microorganisms and the solvents used.

The methanol extracts revealed a high lysozyme potential against two tested Gram-positive bacteria species, *S. agalactiae* and *S. aureus*, with values varying from 0 to 250 U mL^{-1} (Fig. 1). Although the results show that the methanol extracts of *S. cartilaginea* had the highest lysozyme activity against *S. aureus* with 250 U mL^{-1}, they failed against *S. agalactiae.* However, the other three lichen species did have lysozyme activity against *S. agalactiae* and *S. aureus*, but values did not exceed 100 U mL^{-1}.

Lysozyme is an antimicrobial polypeptide that can be used for food preservation; however, its antibacterial potential is limited to Gram-positive bacteria, the lysozyme, which digest bacterial cell walls by breaking (β1 → 4) glycosidic bonds between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). Gram-positive bacteria were more sensitive than Gram-negative ones due to the differences in their cell wall structure.

Extracts from the lichen species have different effects on spore germination of two fungi *Aspergillus niger* and *Penicillium digitatum* tested here (Fig. 2). The percentage of the spore inhibitions was compared with untreated (without extract) samples. The values represented are the germination levels of the spores; to determine the percentage of inhibition these are subtracted from the percentages of the extracts from the untreated samples. Notable spore inhibitions against *A. niger* and *P. digitatum* were obtained with the methanol extract of all four lichens tested. Methanol extracts from *S. cartilaginea* and *X. parietina* showed the highest spore inhibitions against *Penicillium* spp. (59.84% and 43.75%, respectively). The results for *Aspergillus* spore germination showed that the maximum inhibition was obtained with the methanol extracts of *F. caperata* and *X. parietina* (51% and 43.3%, respectively). However, quencher extracts failed to inhibit spore germination of the two fungi. Furthermore, the acetone extracts of all four lichen species have a lower activity against the *Penicillium* and *Aspergillus* species tested (Fig. 2).

As well as demonstrating the effect of lichen extracts on fungal spore germination, methanol extracts, due to their high inhibition of spore germination, were chosen for evaluating its effect on fungal hyphae. The fungal hyphae were more affected by methanol extract from *F. caperata* (0.22 AU/mL/h) and *X.* parietina (0.13 AU/mL/h.), but the two methanol extracts of *D. ocellatus* and *S. cartilaginea* did not affect the hyphal mycelium of *Alternaria* (Fig. 3).

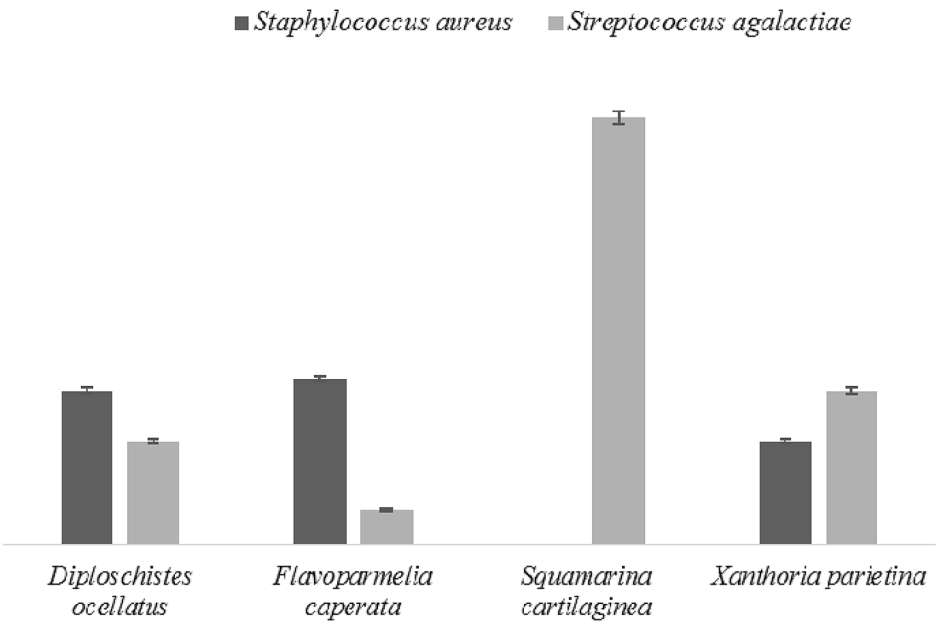

Fig. 1 Lysozyme activity of methanol extracts of *Diploschistes ocellatus*, *Flavoparmelia caperata*, *Squamarina cartilaginea* and *Xanthoria parietina*

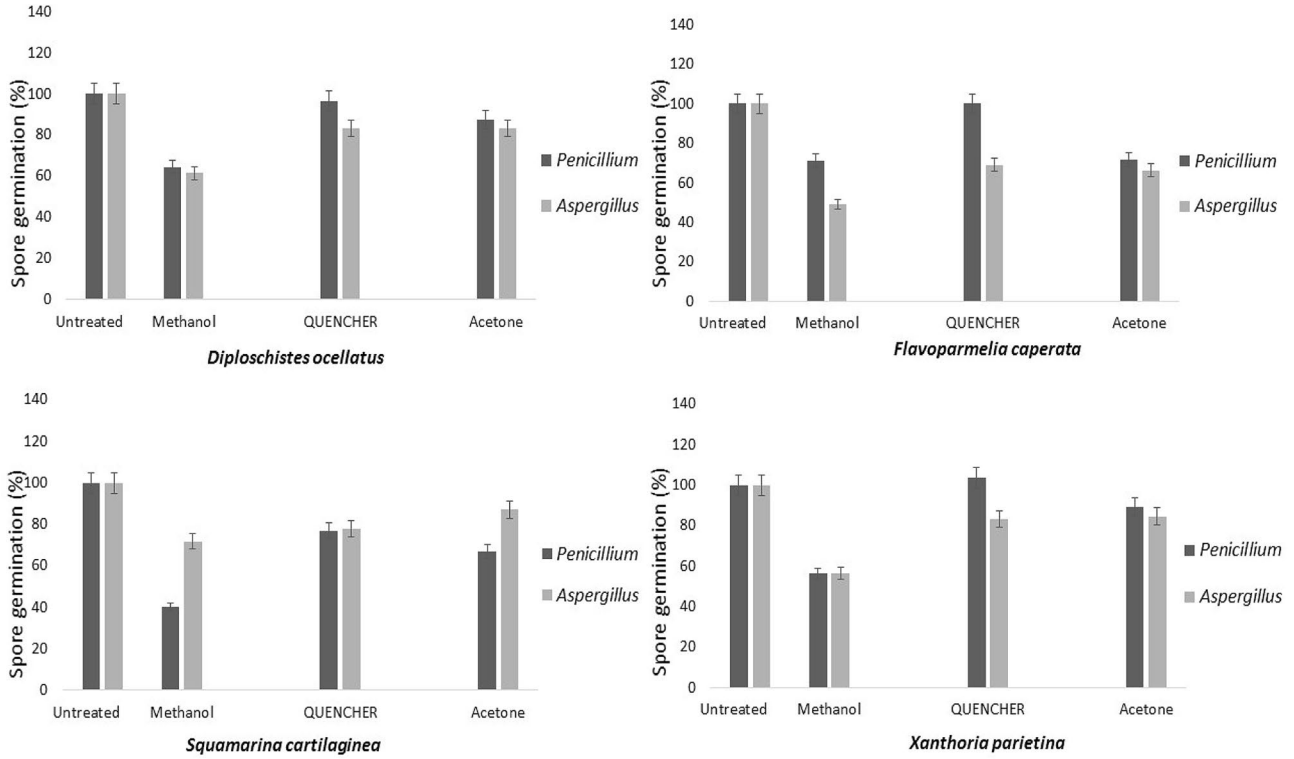

Fig. 2 Impact of methanol, acetone and QUENCHER extracts of *Diploschistes ocellatus*, *Flavoparmelia caperata*, *Squamarina cartilaginea* and *Xanthoria parietina* used at 2000(v/v), on spore germination for *Aspergillus niger* and *Penicillium digitatum.* Three independent experiments were performed and the error bars are given with standard deviation

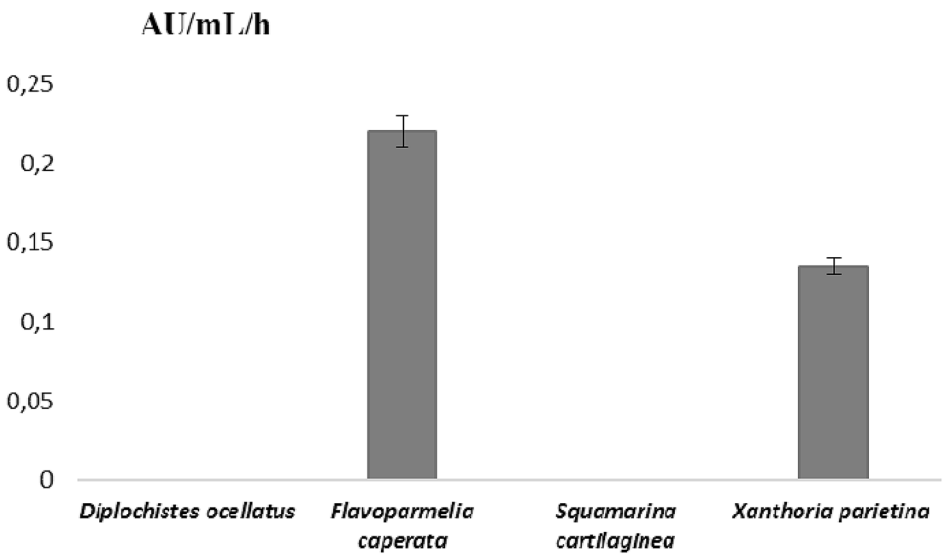

Fig. 3 Effect of methanol extracts from *Diploschistes ocellatus*, *Flavoparmelia caperata*, *Squamarina cartilaginea* and *Xanthoria parietina* on mycelia destruction of *Alternaria alternata* compared to control tube containing mycelium suspension without methanol extract. Values were expressed in AU per volume per hour

Discussion

Due to the increase in bacterial resistance, the emergence of new infectious diseases, and the resurgence of several infections that appeared to have been controlled, many researchers have focused on the investigation of natural products as a source of new bioactive molecules, including those extracted from lichens. Research has clearly shown that lichen extracts have antioxidant, antifungal, antiviral,

anti-inflammatory, insecticidal and antibacterial properties (Ranković 2015). In our study, the antimicrobial potential of extracts taken from *D. ocellatus*, *F. caperata*, *S. cartilaginea* and *X. parietina* collected from Tunisia were evaluated; they were shown to have higher antibacterial activities when compared with, for example, *Heterodermia podocarpa* and *Parmotrema tinctorum* which are effective against *Bacillus licheniformis*, *B. megaterium*, *B. subtilis* and *S. aureus* (Behera et al. 2007). Others reports show that methanol extracts of *Ramalina polymorpha* and *Umbilicaria nylanderiana* have a low inhibition diameter against *E. coli* and are ineffective against *E. cloacae* and *S. aureus* (Gulluce et al. 2006). Almola et al. (2016) showed that an acetone extract of *D. ocellatus* was effective against *Bacillus* sp. and *Micrococcus luteus* (MIC = 50 and 12.5 mg mL^{-1}, respectively). No activity was observed against *E. coli* and *S. aureus* according to Almola et al. (2016), but in our study, an acetone extract of *D. ocellatus* had 125 and 250 µg mL^{-1} MIC value, respectively, against *E. coli* and *S. aureus.* The reasons for the differences between Almola et al. (2016) and our study are most probably due to the use of different lichen concentrations against test bacteria and different bacterial strains.

According to Basile et al. (2015), an acetone extract of *X. parietina* exhibited a good inhibition against *S. aureus*, but Felczykowska et al. (2017) did not find any inhibition; this difference is explained by the latter's work on acetone extracts from in vitro cultured lichen-forming fungi.

Burkholder et al. (1944) tested 42 lichens for their antibiotic properties and achieved the first screening of antimicrobial activity of lichen extracts. Buçukoglu et al. (2012) showed that their antibacterial power is due to the presence of lichen substances. Ranković et al. (2008) demonstrated that lichens are a source of antimicrobial substances, and natural products derived from lichens were proposed as a therapeutic alternative to conventional antimicrobial treatment (Ranković et al. 2015).

Many studies have shown that lichen compounds induce apoptosis in various antibiotic activities; they have a wide range of antibiotic properties, helping, for example, to fight tuberculosis and intestinal infections (Podterob 2008). Furthermore, Kosanić et al. (2012) investigated acetone extracts against different microorganisms by determining the MIC and found that the acetone extract of *F.caperata* exhibited the highest antimicrobial activity, and even at relatively low concentrations inhibited all tested bacteria and fungi. Our work also confirms previous studies that showed significant antimicrobial inhibition by methanol and acetone extracts of *F. caperata* (Aydin and Kinalioğlu 2013; Mitrović et al. 2011).

The results of the antimicrobial behaviour of various lichens extracts (methanol, acetone and quencher) on solid media depend upon the microorganism groups, being more active against bacteria and yeasts, and having no effect on fungal growth. Furthermore, we found that there is a significant difference between the antimicrobial activity of different lichens and that extraction differences depend on the polarity of the solvents employed. The methanol extract of *S. cartilaginea* provided the most effective antimicrobial activity against *E. cloacae*. This is in agreement with Ranković et al. (2007) who noted that the strongest antimicrobial activity was observed with the methanol extracts compared to the extracts in other solvents. Moreover, variations in the antimicrobial activity of various types of lichens and extracts are probably reflecting different quantities of the same active component in lichen extracts, different components involved in antimicrobial activities, different sampling locations of lichens, and different susceptibility of tested microorganisms, as observed by Mitrović et al. (2011).

The use of powdered material without organic solvent, the QUENCHER approach, is a new method for the quantification of phenolic compounds and the measurement of total antioxidant power of foods (Gökmen et al. 2009). This method was employed here for the first time to study antibacterial and antifungal effect using extracts from four lichens derived by means of powdered material. These showed a strong antimicrobial effect against Gram-positive and Gram-negative bacteria, candida and fungal activities by spore germination inhibition. This is also the first report of the impact of these four lichen extracts on lysozyme activity, inhibition of spore germination, and destruction of fungal mycelia.

According to the literature, all the four lichen species studied produce secondary metabolites. *D. ocellatus* with rich in phenolic contents and high antioxidant capacities, were analysed by FTIR and 1H NMR spectroscopy and revealed diversity in terms of chemical composition such as aliphatic acids, aromatic rings and unsaturated compounds (Mendili et al. 2019), and explains their antimicrobial capacity. Dieu et al. (2019) studied antibacterial activity of acetone extract of *F. caperata* and their compounds, especially (+)-usnic acid, and demonstrated a higher inhibition against pathogens. *S. cartilaginea* produces psoromic acid (Huneck and Yoshimura 1996), and Hassan et al. (2019) reported this compound's various biological properties. For *X. parietina*, the parietin is the major compound and is perhaps responsible for the antimicrobial activity, and Basile et al. (2015) noted that this compound from *X. parietina* had antibacterial and antifungal properties. Clearly, the high antimicrobial capacity of *D. ocellatus*, *F. caperata*, *S. cartilaginea* and *X. parietina* attests to the importance of lichens as potential therapeutic agents.

Conclusions

In our study, metabolites extracted from four lichens by methanol, acetone and powdered material clearly demonstrate their antibacterial effect against Gram-positive and Gram-negative bacteria, candida and fungal activities by inhibiting spore germination. The methanol extract contained significant amounts of different antioxidant compounds, and the efficiency of the quencher extract proved to be particularly effective as an anti-candida natural agent.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Almola ZS, Al-Ni'ma BA, Ramadan NA (2016) Antibacterial effect of some Iraqi lichen extracts. IJST 5(9):448–456

Aydin S, Kinalioğlu K (2013) The investigation of antibacterial activities of ethanol and methanol extracts of *Flavoparmelia caperata* (L.) Hale (*Parmeliaceae*) and *Roccella phycopsis* Ach. (*Roccellaceae*) lichens collected from eastern Black Sea Region, Turkey. J App Pharm Sci 3(2):143–147

Basile A, Rigano D, Loppi S, Di Santi A, Nebbioso A, Sorbo S, Bontempo P (2015) Antiproliferative, antibacterial and antifungal activity of the lichen *Xanthoria parietina* and its secondary metabolite parietin. Int J Mol Sci 16(12):7861–7875. https://doi.org/10.3390/ijms16047861

Behera BC, Verma N, Sonone A, Makhija U (2007) Antioxidant and antibacterial properties of some cultured lichens. Bioresource Technol 99:776–784. https://doi.org/10.1016/j.biortech.2007.01.031

Buçukoglu TZ, Albayrak S, Halici MG (2012) Antimicrobial and antioxidant activities of extracts and lichen acids obtained from some *Umbilicaria* species from Central Anatolia, Turkey. J Food Process Preserv 37:1103–1110. https://doi.org/10.1111/j.1745-4549.2012.00811.x

Burkholder PR, Evans AW, Mcveigh I, Thornton HK (1944) Antibiotic activity of lichens. Proc Natl Acad Sci USA 30(9):250–255. https://doi.org/10.1073/pnas.30.9.250

Dias DA, Urban S (2009) Phytochemical investigation of the Australian lichens *Ramalina glaucescens* and *Xanthoria parietina*. Nat Prod Commun 4(7):959–964. https://doi.org/10.1177/1934578x0900400717

Dieu A, Mambu L, Champavier Y, Chaleix V, Sol V, Gloaguen V, Millot M (2019) Antibacterial activity of the lichens *Usnea florida* and *Flavoparmelia caperata* (*Parmeliaceae*). Natl Prod Res. https://doi.org/10.1080/14786419.2018.1561678

Essghaier B, Dhieb C, Rebib H, Ayari S, Rezgui A, Boudabous A, Sadfi-Zouaoui N (2014) Antimicrobial behavior of intracellular proteins from two moderately halophilic bacteria: strain J31 of *Terribacillus halophilus* and strain M3–23 of *Virgibacillus marismortui*. J Plant Pathol Microb 5(1):214. https://doi.org/10.4172/2157-7471.1000214

Felczykowska A, Pastuszak-Skrzypczak A, Pawlik A, Bogucka K, Herman-Antosiewicz A, Guzow-Krzemińska B (2017) Antibacterial and anticancer activities of acetone extracts from in vitro cultured lichen-forming fungi. BMC Complement Altern Med 17(1):300. https://doi.org/10.1186/s12906-017-1819-8

Ganesan A, Thangapandian M, Ponnusamy P, Sundararaj JP, Nayaka S (2015) Antioxidant and antibacterial activity of parmeliod lichens from Shevaroy hills of Eastern Ghats. India Int J PharmTech Res 8(9):13–23

Gökmen V, Serpen A, Fogliano V (2009) Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER' approach. J Food Sci Technol 20:278–288. https://doi.org/10.1016/j.talanta.2013.02.061

Gomez-Serranillos MP, Fernández-Moriano C, González-Burgos E, Divakar PK, Crespo A (2014) *Parmeliaceae* family: phytochemistry, pharmacological potential and phylogenetic features. Royal Soc Chem Adv 4:59017–59047. https://doi.org/10.1039/C4RA09104C

Graciela M, Vignolo M, Kairuz N, Aida AP, Ruiz H, Oliver G (1995) Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by *Lactobacillus casei* CRL 705. J Appl Microbiol 78:5–10. https://doi.org/10.1111/j.1365-2672.1995.tb01665.x

Gulluce M, Aslan A, Sokmen M, Sahin F, Adiguzel A, Agar G, Sokmen A (2006) Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca, Ramalina pollinaria, Ramalina polymorpha* and *Umbilicaria nylanderiana*. Phytomedicine 13:515–521. https://doi.org/10.1016/j.phymed.2005.09.008

Gupta VK, Darokar MP, Saikia D, Pal A, Fatima A, Khanuja SPS (2007) Antimycobacterial activity of lichens. Pharm Biol 45(3):200–204. https://doi.org/10.1080/13880200701213088

Hassan STS, Šudomová M, Berchová-Bímová K, Šmejkal K, Echeverría J (2019) Psoromic acid, a lichen-derived molecule, inhibits the replication of HSV-1 and HSV-2, and inactivates HSV-1 DNA polymerase: shedding light on antiherpetic properties. Molecules 24(16):2912. https://doi.org/10.3390/molecules24162912

Huneck S, Yoshimura I (1996) Identification of lichen substances. Springer-Verlag, Berlin

Jorgensen JH, Turnidge JD (2007) Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (eds) Manual of clinical microbiology, 9th edn. American Society for Microbiology, Washington, pp 1152–1172

Kosanić M, Ranković B, Stanojković T (2012) Antioxidant, antimicrobial, and anticancer activities of three *Parmelia* species. J Sci Food Agric 9:1909–1916. https://doi.org/10.1002/jsfa.5559

Mendili M, Bannour M, Araújo MEM, Aschi-Smiti S, Seaward MRD, Khadhri A (2019) Secondary metabolites and antioxidant capacity of the Tunisian lichen *Diploschistes ocellatus* (*Ascomycota*). Int J Med Mushrooms 21(8):817–823. https://doi.org/10.1615/IntJMedMushrooms.2019031423

Mitrović T, Stamenković S, Cvetković V, Tošić S, Stanković M, Radojević I, Stefanović O, Čomić L, Đačić D, Ćurčić M, Marković S (2011) Antioxidant, antimicrobial and antiproliferative activities of five lichen species. Int J Mol Sci 12(8):5428–5448. https://doi.org/10.3390/ijms12085428

Nugraha AS, Untari LF, Laub A, Porzel A, Franke K, Wessjohann LA (2020) Anthelmintic and antimicrobial activities of three new depsides and ten known depsides and phenols from Indonesian lichen: *Parmelia cetrata* Ach. Nat Prod Res. https://doi.org/10.1080/14786419.2020.1761361

Podterob AP (2008) Chemical composition of lichens and their medical applications. Pharm Chem J 42:582–588

Ranković B (2015) Lichen secondary metabolites. Bioactive properties and pharmaceutical potential. Springer International, Switzerland

Ranković B, Misić M, Sukdolak S (2007) Antimicrobial activity of extracts of the lichens *Cladonia furcata*, *Parmelia caperata*,

Parmelia pertusa, *Hypogymnia physodes* and *Umbilicaria polyphylla*. Br J Biomed Sci 64:143–148

Ranković B, Misić M, Sukdolak S (2008) The antimicrobial activity of substances derived from the lichens *Physcia aipolia, Umbilicaria polyphylla, Parmelia caperata* and *Hypogymnia physodes*. World J Microbiol Biotechnol 24:1239–1242. https://doi.org/10.1007/s11274-007-9580-7

Ryazanova LP, Stepnaya OA, Suzina NE, Kulaev IS (2005) Antifungal action of the lytic enzyme complex from *Lysobacter* sp. XL.1. Process Biochem 40:557–564. https://doi.org/10.1016/j.procbio.2004.01.031

Sarangi N, Athukorala P, Fernando D, RashidKievit KYTD (2010) The role of volatile and non-volatile antibiotics produced by *Pseudomonas chlororaphis* strain PA23 in its root colonization and control of *Sclerotinia sclerotiorum*. Biocontrol Sci Technol 20:875–890. https://doi.org/10.1080/09583157.2010.484484

Soundararajan S, Shanmugam P, Nagarajan N, Palanisamy D, Ponnusamy P (2019) In vitro study on screening antimicrobial and anti-oxidant potential of *Ramalina fastigiata*. J Drug Deliv Ther 9(1):216–219. https://doi.org/10.22270/jddt.v9i1.2217

Stocker-Wörgötter E (2008) Metabolic diversity of lichen-forming ascomycetous fungi: culturing, polyketide and shikimate metabolite production, and PKS genes. Nat Prod Res 25:188–200. https://doi.org/10.1039/b606983p

Tas I, Yildirim AB, Ozkan E, Ozyigitoglu GC, Yavuz MZ, Turker AU (2019) Biological evaluation and phytochemical profiling of some lichen species. Acta Aliment 48(4):457–465. https://doi.org/10.1556/066.2019.48.4.7

Thadhani VM, Choudhary MI, Ali S, Omar I, Siddique H, Karunaratne V (2011) Antioxidant activity of some lichen metabolites. Nat Prod Res 25:1827–1837. https://doi.org/10.1080/14786419.2010.529546

Zheng KX, Jiang Y, Jiang JX, Huang R, He J, Wu SH (2019) A new phthalazinone derivative and a new isoflavonoid glycoside from lichen-associated *Amycolatopsis* sp. Fitoterapia 135:85–89. https://doi.org/10.1016/j.fitote.2019.04.011