NATURAL PRODUCT RESEARCH
2022, VOL. 36, NO. 12, 3095–3099
https://doi.org/10.1080/14786419.2021.1933974

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SHORT COMMUNICATION

Does the lichenicolous fungus *Heterocephalacria bachmannii* affect the antimicrobial potential of its host *Cladonia foliacea*?

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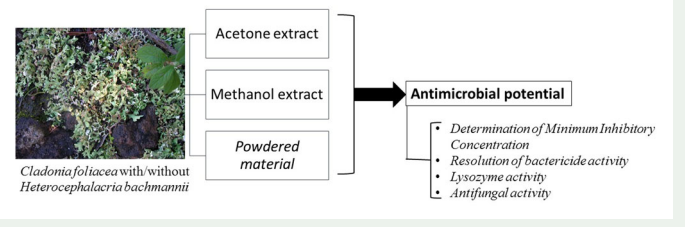
ABSTRACT

The effects of the lichenicolous fungus *Heterocephalacria bachmannii* on the antimicrobial potential of the lichen *Cladonia foliacea* demonstrated that the extracts investigated have antimicrobial potential against gram-positive and negative bacteria, and yeast, and inhibit the germination of fungal spores. Inhibition activity varied considerably depending on the extract, the bacterial species, and the absence or presence of *H. bachmannii*; unparasitised *C. foliacea* has a higher antimicrobial activity. Methanol and acetone extracts of *C. foliacea* alone have higher inhibition diameters than *C. foliacea* with *H. bachmannii* against *Enterobacter cloacae*; the methanol extract of *C. foliacea* showed the best inhibition (250 µg/ml). *C. foliacea* also has a high lysozyme potential against *Streptococcus agalactiae* and *Staphylococcus aureus*. Fungal hyphae of *Alternaria alternata* were more affected by the methanol extract from *C. foliacea*.



ARTICLE HISTORY
Received 1 February 2021
Accepted 17 May 2021

KEYWORDS
Antimicrobial potential; Cladonia foliacea; Heterocephalacria bachmannii; lichenicolous fungi; lysozyme activity; QUENCHER

1. Introduction

The pharmaceutical, biological and ecological benefits of secondary compounds in lichens has a history of more than 100 years (Goga et al. 2020). Secondary metabolites from lichens have attracted increased attention for their potential to produce chemicals that differ from those in plants (Ranković 2015), c. 1,000 of which are specific to lichens (Goga et al. 2020). Secondary metabolites of lichens have a wide variety of

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.1933974.

biological activity (Ranković 2015; Mendili et al. 2021) and are a potential source of antibacterial drugs (Shrestha et al. 2014).

As well as being a symbiotic system, lichens are also recognised as an ecosystem for other organisms, including lichenicolous fungi which are parasitic on lichen thalli (Kellogg and Raja 2017). Lichenicolous fungi are also a valuable source of various bioactive chemicals such as phenolic acids, alkanes and aromatic compounds (Kellogg and Raja 2017; Khadhri et al. 2019); one such example is *H. bachmannii* hosted by *C. foliacea* (Khadhri et al. 2019) which synthesises many secondary metabolites, including usnic acid, atranorin and fumarprotocetraric acid, as well as antibacterial properties (Yılmaz et al. 2004). Khadhri et al. (2019) showed that the chemical analysis of the acetone and methanol extracts of *C. foliacea* with and without *H. bachmannii* by FTIR and NMR-H produced a high diversity of chemical compounds, the most active of which were phenolic acids, alkanes and aromatic compounds. Furthermore, they revealed that *C. foliacea* with and without *H. bachmannii* had a significant phenolic content and antioxidant potential.

The purpose of the present work was to determine their antimicrobial potential *in vitro*, in addition to their minimal inhibitory concentration (MIC), lysozyme and antifungal effects of *C. foliacea* with and without *H. bachmannii* by means of extracts in Tunisian material against gram-negative and gram-positive bacteria, yeasts and fungi.

2. Results and discussion

The antimicrobial potential of extracts (methanol, acetone and QUENCHER) from *C. foliacea* + *H. bachmannii* versus *C. foliacea* against gram-negative bacteria (*Enterobacter cloacae* & *Escherichia coli*), gram-positive bacteria (*Staphylococcus aureus* & *Streptococcus agalactiae*), yeasts (*Candida albicans, C. sake* & *C. parapsilosis*) and fungi (*Penicillium* spp., *Aspergillus* spp., *Alternaria alternata* & *Colletotrichum acutatum*) was determined. Estimations of the noticeable antibacterial activities of these extracts were based on the presence or absence of inhibitory zones (Table S1).

The majority of the *C. foliacea* + *H. bachmannii* extracts when compared with *C. foliacea* extracts were found to be active against all the pathogens tested, the inhibition ranging from 10 to 31 mm. The highest inhibition diameters observed with the methanol and acetone extracts of *C. foliacea* against *E. cloacae* (31 mm and 29 mm, respectively) followed by methanol extract of *C. foliacea* against *E. coli* (27.5 mm). However, *C. foliacea* + *H. bachmannii* showed a diameter of 16.75 mm against *E. cloacae* with the methanol extract and 14 mm against *E. coli* with the acetone extract, and the inhibition diameter of *Streptococcus* species was only 13 mm with the methanol extract.

For anti-yeast activity, the methanol extracts of *C. foliacea* + *H. bachmannii* exhibited a high inhibition diameter (18 mm) against *Candida albicans* followed by 15 mm for the QUENCHER extract of *C. foliacea* against *C. albicans*.

MIC are reported in Table S2. The MIC values vary from 250 to 2000 µg/ml. The best antibacterial activity was shown by the methanol extract of *C. foliacea* against *Enterobacter cloacae* (250 µg/ml). While the highest antibacterial activity of *C. foliacea* + *H. bachmannii* was found with the acetone extract against *E. cloacae* (500 µg/ml). Of

the *Candida* spp. investigated, the acetone and methanol extracts of *C. foliacea* + *H. bachmannii* show the best MIC against *C. albicans* (500 μg/ml). The methanol extracts of *C. foliacea* + *H. bachmannii* inhibited *C. parapsilosis* (MIC 500 μg/ml), and the QUENCHER extracts of *C. foliacea* inhibited *C. albicans* (500 μg/mL).

There were significant differences ($p < 0.05$) in the lysozyme activity. The methanol extracts of *C. foliacea* revealed a high lysozyme activity against *S. agalactiae* and *S. aureus,* with values 18 and 35 AU mL^{-1}. However, the methanol extract of *C. foliacea* + *H. bachmannii* revealed a lysozyme potential of 10 AU mL^{-1} against *Staphylococcus aureus,* but it was unable to inhibit *S. agalactiae* (Figure S1).

Figure S2 illustrates the effects of extracts from *C. foliacea* + *H. bachmannii*, and from *C. foliacea* on the spore germination of the fungi *Aspergillus niger* and *Penicillium digitatum*. Untreated (without extract) was compared with the percentage of spore inhibitions (Figure S2). Methanol extracts of *C. foliacea* + *H. bachmannii* exhibited the highest effect (53.59%) against *P. digitatum*. and for *C. foliacea* this was 46.45% against *A. niger*, while the acetone extract of *C. foliacea* inhibited *A. niger*. and *P. digitatum* by 41.1% and 26.25%, respectively. Acetone extracts of *C. foliacea* + *H. bachmannii* showed low inhibition, and QUENCHER extracts failed to inhibit spore germination of these two fungi (Figure S2).

Figure S3 shows the effect of *C. foliacea* + *H. bachmannii* extracts on mycelial destruction of *Alternaria alternata*. Fungal hyphae were more affected by methanol extract from *C. foliacea* with 0.095 AU/mL/H, but was powerless when *C. foliacea* was parasitised (0.046 AU/mL/H).

The antibiotic properties of lichens were first reported by Burkholder et al. (1944), since when many studies have investigated the antibacterial activity of a relatively small number of lichen species against various gram-positive and gram-negative bacteria (Mendili et al. 2021). Lichenicolous fungi that parasitise lichen thalli have also been shown to produce various bioactive compounds (Kellogg and Raja 2017; Khadhri et al. 2019).

C. foliacea, the host for *H. bachmannii*, showed high antibacterial activities similar to other *Cladonia* species, such as *C. furcata* and *C. rangiferina* which are inhibitors of *Echerchia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* (Kosanić et al. 2014). Shrestha et al. (2014), who investigated acetone and methanol extracts of 34 lichen species against four bacterial strains by determining the MIC, found that most of the lichen extracts demonstrated inhibitory effects against *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and methicillin-resistant *S. aureus*. Differences in antibacterial activity for lichen species are due to the presence of their different chemical compounds (Kosanić et al. 2014).

C. foliacea with and without *H. bachmannii*. produces secondary metabolites which are rich in phenolic contents and have high antioxidant capacity; FTIR and H NMR spectroscopy revealed the presence of, for example, phenolic acids, alkanes and aromatic compounds (Khadhri et al. 2019). *Cladonia* species that are hosts for *H. bachmannii* also synthesise a variety of secondary metabolites including aliphatic acids, depsides, depsidones, the dibenzofuran and terpenoid (Ranković et al. 2015); these compounds had previously been shown to have powerful antibacterial activity as noted by Kosanić et al. (2014). Yılmaz et al. (2004) showed that *Cladonia foliacea*

extracts and their compounds usnic acid, atranorin and fumarprotocetraric acid were effective against *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Proteus vulgaris*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Candida albicans* and *C. glabrata*.

The current study confirms the antimicrobial potential of *C. foliacea* extract in previous studies (Yılmaz et al. 2004). However, this is the first study reporting the antimicrobial effects of extracts from *C. foliacea* with its parasite *H. bachmannii*. We conclude that *C. foliacea* when parasitised by *H. bachmannii* has low antimicrobial and lysozyme properties; the fungal hyphae of *Alternaria alternata* were less affected, but a positive effect on the spore germination against *A. niger* proved that *H. bachmannii* induced an antagonistic effect in combination with the host species.

3. Experimental

Supplementary material relating to this article is available online, alongside Figures S1, S2, S3 and Tables S1, S2.

4. Conclusions

The antimicrobial activities of *C. foliacea* + *H. bachmannii* and *C. foliacea* extracts vary according to the species and solvents used for the extractions, as well as the bacteria tested (gram-positive and gram-negative bacteria) and yeast. Our results show that in the presence of the parasitic fungus, *C. foliacea* showed a high anti-yeast activity and positive effect on spore germination against *P. digitatum* that demonstrates a synergic effect. However, when *C. foliacea*, and the fungal hyphae of *A. alternata* were more affected and have a positive effect on the spore germination against *A. niger* which reveals an antagonistic effect of *H. bachmannii*.

Disclosure statement

No conflict of interest was reported by the authors.

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