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SHORT COMMUNICATION

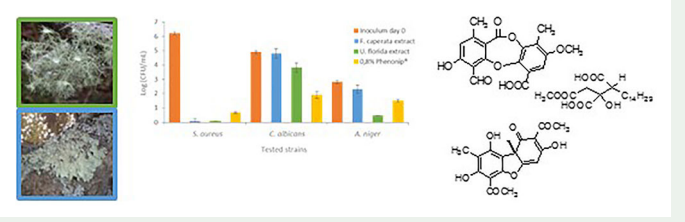
Antibacterial activity of the lichens *Usnea Florida* and *Flavoparmelia caperata* (Parmeliaceae)

Amandine Dieu[a], Lengo Mambu[a], Yves Champavier[b], Vincent Chaleix[a], Vincent Sol[a], Vincent Gloaguen[a] and Marion Millot[a]

[a]Laboratoire PEIRENE (EA 7500), Universit é de Limoges, Limoges cedex, France; [b]BISCEM Plateform, Université de Limoges, Limoges cedex, France

ABSTRACT

Acetone extracts of the two common epiphytes lichens *Usnea florida* and *Flavoparmelia caperata* have been evaluated for their antimicrobial activities against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus brasiliensis*. The dibenzofuran derivative (+)-usnic acid (**1**) was the main metabolite in these two species. Thamnolic (**5**), evernic (**6**), physodic (**7**) and 3-hydroxyphysodic acids (**8**) were isolated from *U. florida*, as well as 5,7-dihydroxy-6-methyl-phtalide (**2**) which was newly identified in this *Genus*. Protocetraric (**3**) and caperatic acids (**4**) and ergosterol peroxide (**9**) are usually biosynthezised by *F. caperata*. Antibacterial activity was determined for the four main compounds against *Staphylococcus aureus* using bioautography and broth dilution method. Minimal inhibitory concentrations of usnic acid, caperatic acid and protocetraric acid were comprised between 7.25 and 12.5 μg/mL.



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1. Introduction

Lichens result from symbiosis between a fungus and a photosynthetic organism, either an alga and/or a cyanobacterium (Stocker-Wörgötter 2008). Lichen secondary metabolites such as depsides, depsidones, dibenzofurans or xanthones, have been shown to exhibit antibiotic, antifungal, antiviral, antioxidant, or antipyretic properties (Pandey 2017).

We report herein results on two common lichen species belong to the Parmeliaceae: *Usnea florida* (L.) Weber ex F.H. Wigg and *Flavoparmelia caperata* (L.) Hale, widely present on trees of central French forests. *Usnea florida* is reported to be

CONTACT Marion Millot marion.millot@unilim.fr
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use as a traditional medicine in various countries (Prateeksha et al. 2016). Usnic acid is present in these two species and is responsible for the yellow-green color of the thallus (Smith 2009). The aim of this study was to isolate compounds which exhibit antibacterial activity for use as preservatives, in order to find an alternative to parabens in cosmetic industry (DIRECTIVE 2003). The present bioassay-guided fractionation of *U. florida* and *F. caperata* acetone extracts led to the isolation and identification of the widely spread secondary lichen metabolite (+)-usnic acid (**1**) together with eight known compounds (**2-9**). Compound (**2**), 5,7-dihydroxy-6-methylphtalide, has been newly identified in the Genus *Usnea*.

2. Results and discussion

A protocol adapted from the Challenge Test was used to evaluate antimicrobial activity of lichen acetone extracts. Extracts were evaluated against five strains of common microbes: *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Escherichia coli*, *Candida albicans* and *Aspergillus brasiliensis*. Results did not show any effect against Gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. Acetonic extracts displayed a moderate fungicidal activity against *Candida albicans* and *Aspergillus brasiliensis* (Figure 1).

At day 7, 0.1% of the acetonic extract of *U. florida* in solution showed activity against *A. brasiliensis* three fold better than that exerted by Phenonip®, a preservative consisting of a mixture of parabens, used in the cosmetic industry. A weak activity is observed for *U. florida* acetonic extract against *C. albicans* at day 7 with a solution at 0.1% while no activity is observed for the other tested concentrations. A weak fungicidal activity is observed for acetonic extract of *F. caperata* against *A. brasiliensis* while no activity was observed against *C. albicans* growth. Strong antibacterial activity is observed for the two extracts with a better inhibition of *S. aureus* growth at day 7 with a solution at 0.1%. These results are consistent with other studies (Ranković et al. 2007; Kosanić et al. 2012; Ranković et al. 2012), which have shown that Gram positive

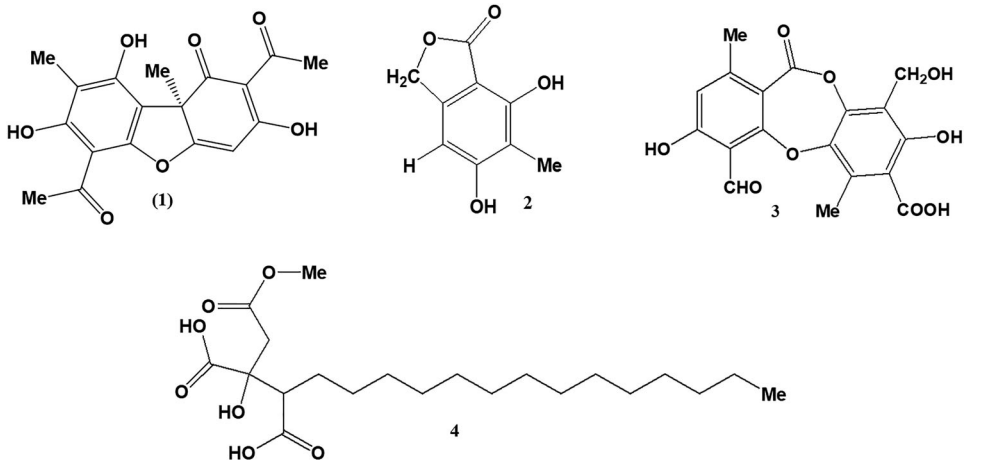

Figure 1. Structure of compound 1-4: usnic acid (**1**), 5,7-dihydroxy-6-methylphtalide (**2**) protocetraric acid (**3**) and caperatic acid (**4**).

bacteria are more sensitive than fungi because of differences in composition and permeability of the cell wall. In order to determine the minimal inhibitory concentrations (MIC), the extracts have been tested against *S. aureus* at four concentrations: 25, 30, 35 and 40 μg/mL. For *F. caperata* acetone extract, the MIC rich 40 μg/mL with 99.5% of inhibition of the bacteria growth. For *U. florida*, the MIC is determined at 30 μg/mL. This result differ from the observation of Cankilic et al (Cankiliiô et al. 2017) and the weak value obtained for the MIC of acetonic extract show the biological interest of the chemotype studied here (Bate et al. 2018).

Following these preliminary results, a TLC-bioautographic protocol was performed against *S. aureus* in order to detect active compounds of *U. florida* and *F. caperata* acetone extracts. After revelation by MTT, two clear inhibition zones named B and C, corresponding to active compounds, were observed. The higher inhibition zone is identified as usnic acid in comparison with standard (Phe = Phenonip). Thus, usnic acid content has been evaluated by HPLC in the two species in order to establish a potential correlation between the activity of the extract and the presence of usnic acid. It represents 1.4% of dry wt of *F. caperata* (20% of the extract) and 1.1% of dried wt of *U. florida* (44% of the extract). Despite a large difference in the quantity of usnic acid in the two extracts, their activity against *S. aureus* is similar. Usnic acid alone is therefore not responsible for the activity of the extracts on the microorganisms tested, which suggests a cumulative or synergistic action with other compounds.

To identify these other compounds responsible for the antibacterial activity, bioassay-guided fractionation of the two lichen extracts has been done. *U. florida* acetonic extract was chromatographed using a C_{18}-silica gel MPLC followed by preparative TLCs. Six known compounds have been identified: the dibenzofuran (+)-usnic acid (**1**), two depsides (thamnolic and evernic acids), two depsidones (physodic and 3-hydroxyphysodic acids) and 5,7-dihydroxy-6-methylphtalide (**2**). Thamnolic and usnic acids are the two major compounds of the extract and represent 50% of the dry weigh of the extract. Four minor compounds have been reported for the first time in this chemotype: evernic, physodic, hydroxyphysodic acids and 5-7-dihydroxy-6-methylphtalide. Compound **2** is rarely found in lichens; it has been previously detected in a saxicolous lichen, *Calvitimela armeniaca* (Hertel and Andreev 2003) and isolated from *Anamylospora pulcherrina* (Huneck and Elix 1993). The results corroborated the existence of chemotype in Europe containing usnic and thamnolic acids. Bioassay-guided fractionation of *F. caperata* acetonic extract led to the isolation of ergosterol peroxide, described here for the first time in this species, and two bioactive compounds: protocetraric (**3**) and caperatic acids (**4**) (Sala and Sargent 1981; Huneck and Yoshimura 1996).

In order to confirm the implication of the isolated compounds in the antibacterial activity of the extracts, TLC-bioautography has been carried out on (+)-usnic acid (**1**), physodic acid, 3-hydroxyphysodic acid and caperatic acid (**4**) which exert an inhibition of *S. aureus* growth.

These compounds were found to exhibit activity against *S. aureus*. The high activity of (+)-usnic acid against this species is well known (Lauterwein et al. 1995). Physodic acid and its derivative 3-hydroxyphysodic acid have also already been evaluated for their antibacterial activity against *S. aureus* (Yilmaz et al. 2005; Türk et al. 2006). But to

our knowledge, biological activity of caperatic acid has not been previously described in the literature and its antibacterial activity against *S. aureus* is presented here for the first time. Our results showed that the antibacterial activity of *U. florida* and *F. caperata* acetone extract against *S. aureus* can be attributed to the combined activities of the different compounds described above.

In order to determine MICs against *S. aureus*, a broth microdilution assay was performed. *U. florida* and *F. caperata* acetone extracts display MIC $> 40\,\mu g/mL$ while pure compounds (+)-usnic acid (**1**), protocetraric acid (**3**), caperatic acid (**4**) display MIC of 7.5, 12.5 and 10.0 μg/mL respectively. Phenonip® was used as a positive control.

Protocetraric acid is known to possess a wide antimicrobial spectrum (Prateeksha et al. 2016) and its activity against *S. aureus* has already been described (Manojlović et al. 2012). There is no information in the literature concerning the MIC of caperatic acid against *S. aureus*. Unlike the results obtained by TLC-bioautography, protocetraric acid showed a good bactericidal activity better than Phenonip® (positive control) by the broth microdilution assay, which is a more sensitive and accurate method. They could be good candidates to replace parabens in cosmetics and in skin care products.

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Conflict of interest

The authors declare no conflict of interest.

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