AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Characterization and antimicrobial activity of usnic acid from tropical lichens collected from Doi Suthep Pui, Thailand

Tanat Tilapho[a], Suttinee Kaewsriprakai[a], Vasun Poengsungnoen[b], Kawinnat Buaruang[b], Wetchasart Polyiam[b], Chaisak Chansriniyom[c,d], Ariya Khanboon[e], Chatchai Chaotham[e], Wongsakorn Phongsopitanun[e], Trinset Weeraphan[e,*]

[a] *Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand.*

[b] *Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand*

[c] *Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*

[d] *Natural Products and Nanoparticles Research Unit (NP2), Chulalongkorn University, Bangkok 10330, Thailand*

[e] *Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330 Thailand*

Article Info

Article history:
Received 13 September 2024
Revised 25 January 2025
Accepted 8 February 2025
Available online 25 April 2025

Keywords:
Antimicrobial activity,
Lichen secondary metabolites,
Methicillin-resistant *Staphylococcus aureus* (MRSA),
Natural product,
Usnic acid

Abstract

Importance of the work: Lichens, as symbiotic organisms, are a rich and underexplored source of natural products with the potential to address the urgent need for new antimicrobials against drug-resistant pathogens.

Objectives: To investigate antimicrobial properties of tropical lichen extracts, focusing on solvent extraction for usnic acid and other bioactive compounds.

Materials and Methods: In total, 14 lichen samples were collected from Doi Suthep Pui, Thailand. Secondary metabolites were extracted using chloroform and methanol. Antimicrobial activity was assessed based on agar disc diffusion against six bacterial strains and one yeast. Thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR) spectroscopy were used to identify and purify bioactive compounds such as usnic acid.

Results: The chloroform extracts had higher antimicrobial activity than the methanol extracts. Usnic acid was detected in the chloroform extracts of samples PL05 and PL11−PL14 using TLC, with the yields of purified usnic acid from PL11−PL14 confirmed using NMR spectroscopy. Inhibition zone diameters against methicillin-resistant *Staphylococcus aureus* (MRSA) were in the range 12.5–19.0 mm for these samples. Notably, PL08 and PL09, without detectable usnic acid, still demonstrated strong antimicrobial activity, suggesting the presence of other bioactive compounds. Crude extract yields varied, with methanol producing higher amounts (16.4 mg/g for PL06) than chloroform (2.8 mg/g for PL01). The highest antimicrobial activity was observed against *K. rhizophila*, indicating susceptibility to lichen-derived compounds. These results underscored the potential of lichen extracts, particularly usnic acid, as antimicrobial agents.

Main finding: Usnic acid and other unidentified compounds from tropical lichens exhibited strong antimicrobial activity, particularly against MRSA, which highlighted the potential of lichen-derived bioactive compounds for developing novel antimicrobial agents against drug-resistant pathogens.

* Corresponding author.
E-mail address: trinset02@gmail.com (T. Weeraphan)

online 2452-316X print 2468-1458/Copyright © 2025. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/), production and hosting by Kasetsart University Research and Development Institute on behalf of Kasetsart University.

https://doi.org/10.34044/j.anres.2025.59.2.08

Introduction

Natural products have long been essential in drug discovery, with microbes serving as a primary source of these potent bioactive compounds (Atanasov et al., 2021). However, the emergence of drug-resistant pathogenic bacteria presents a major challenge to public health, creating an urgent need for new antibiotics and antimicrobial agents. Natural products offer a promising solution to this critical problem due to their diverse chemical structures and unique mechanisms of action. Among the diverse sources of natural products, lichens stand out as particularly valuable, representing a symbiotic association between mycobionts (fungi) and photobionts (algae or cyanobacteria) and are distributed worldwide (Tagirdzhanova et al., 2023). Traditionally, these organisms have been used in medicine, functional food and national dyes, with their pharmacological potential increasingly recognized in modern research (Ivanova and Ivanov, 2009; Rassabina et al., 2022). The unique symbiotic relationship allows lichens to produce a diverse array of secondary metabolites, many of which have been found to have major biological activities with not only antibiotic potential, but a wide range of potential pharmacological applications such as antioxidant, antifungal, antiviral and cytotoxic substances (Ren et al., 2023).

Usnic acid is a notable bioactive chemical derived from dibenzofuran obtained from lichens (mostly found in the *Usnea* genus but also from some other genera of lichens) and is known for its wide range of biological activity (Ingólfsdóttir, 2002). There have been extensive studies on its antimicrobial, antiviral, antiprotozoal, anti-inflammatory and anticancer properties (Maulidiyah et al., 2020). Its unique structure and bioactivity make usnic acid highly desirable for isolation in pharmaceutical research.

Thailand has a remarkably high lichen diversity, with 1,292 lichen species recorded, including, 17 species belong to the genus *Usnea*: *U. australis*, *U. baileyi*, *U. baileyi* subsp. *chrysopora*, *U. baileyi* subsp. *septentrionalis*, *U. cinchonarum*, *U. dasycera*, *U. endochroa*, *U. endochroa* var. *papillata*, *U. endorhodina*, *U. florida*, *U. florida* var. *subcomosa*, *U. himantodes*, *U. hossei*, *U. leucospilodea*, *U. luridorufa*, *U. neoguineensis* var. *neoguineensis*, *U. pectinata*, *U. perplexans* and *U. rubicunda* (Buaruang et al., 2017). The rich lichen biodiversity in Thailand provides a valuable resource for isolating bioactive compounds such as usnic acid. Given the considerable pharmaceutical potential of these compounds, there is a pressing need to systematically explore and isolate usnic acid and other lichen-derived compounds from lichens collected in Thailand. Therefore, the present aimed to extract secondary metabolites from 14 lichens collected during an investigation of lichen diversity in Doi-Suthep Pui National Park, Thailand. Each lichen was evaluated for its antibiotic properties and characterized for its bioactive compounds. In addition, the biological activities of usnic acid were investigated.

Materials and Methods

Lichen sample collection

In total, 14 lichen samples were collected from Doi Suthep Pui National Park, Thailand (Fig. 1). Each lichen sample was assigned with a unique code, placed in a separate paper box and transported to the laboratory. Subsequently, the samples were air-dried on a clean bench for 5 d before being used in further studies.

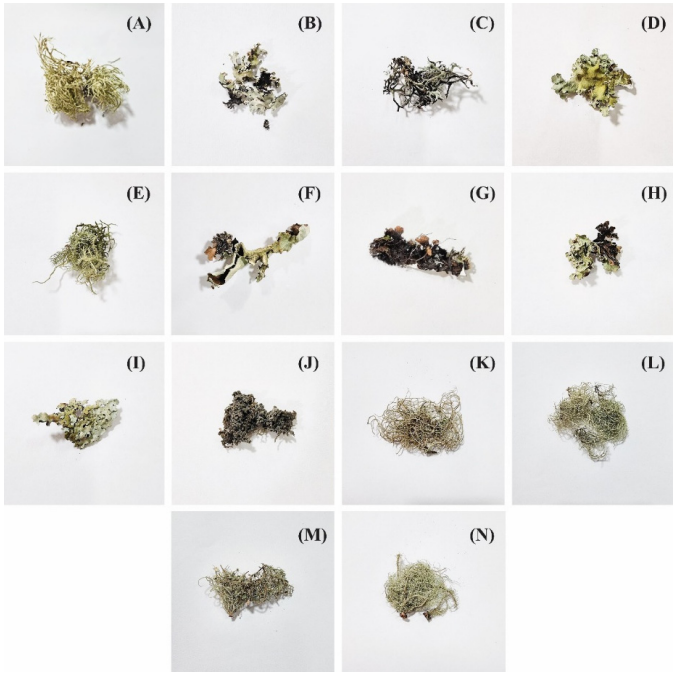


Fig. 1 Lichen samples collected: (A) *Ramalina conduplicans* Vain. (PL01); (B) *Parmotrema reticulatum* (Taylor) M. Choisy (PL02); (C) *Hypotrachyna vexans* (Zahlbr. ex W.L. Culb. & C.F. Culb.) Divakar, A. Crespo, Sipman, Elix & Lumbsch (PL03); (D) *Parmotrema reticulatum* (Taylor) M. Choisy (PL04); (E) *Usnea undulata* Stirt (PL05); (F) *Parmotrema tinctorum* (Despr. ex Nyl.) Hale (PL06); (G) *Parmotrema reticulatum* (Taylor) M. Choisy (PL07); (H) *Parmotrema reticulatum* (Taylor) M. Choisy (PL08); (I) *Hypotrachyna physcioides* (Nyl.) Hale (PL09); (J) *Leptogium gelatinosum* (With.) J.R. Laundon (PL10); (K) *Usnea rubicunda* (PL11); (L) *Usnea undulata* (PL12); (M) *Usnea subflorida* (PL13); (N) *Usnea baileyi* (PL14)

Lichen extract preparation

The lichen secondary metabolites were extracted from the lichen samples using a maceration technique. A sample (1 g) of each lichen was subjected to two separate extractions (one with chloroform and the other with methanol), maintaining a 4:1 solvent-to-sample ratio for each extraction. These solvents were macerated into the respective sample, which was then allowed to stand for 24 hr to facilitate the extraction of metabolites. After maceration, the lichen thalli were separated from each solvent using paper filtration. Then, the filtrates were evaporated under reduced pressure to dryness, yielding crude lichen extracts. The crude extracts were dissolved in chloroform at a concentration of 10 mg/mL for analysis using thin-layer chromatography (TLC) on Silica gel 60 F254 plates. The mobile phase was composed of ethyl acetate and hexane (1:1). (+)-Usnic acid (Sigma-Aldrich, Merck, Germany), with a retention factor (R_f) value of 0.77, was used as the chemical standard.

Antimicrobial activity

Agar disc diffusion was used to assess the antimicrobial activity of lichen extracts. The test microorganisms six bacterial strains—*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20646, *Bacillus subtilis* ATCC 6633, *Streptococcus mutans* ATCC 25175 and *Kocuria rhisozphila* ATCC 9341—along with one yeast strain, *Candida glabrata* TISTR 5006. All bacteria were cultured on tryptic soy agar (TSA) at 37°C for 24 hr, while the yeast was cultured on Sabouraud dextrose agar (SDA) at 30°C for 48 hr. Inoculum preparation involved suspending the microorganisms in 0.85% (weight per volume) normal saline solution, adjusting the turbidity to match the McFarland 0.5 standard and swabbing each suspended bacterium and yeast onto TSA and SDA agar, respectively. For the assay, 20 µL of each lichen extract was applied to sterile paper discs (6 mm diameter) and air-dried on a clean bench. Next, these discs were placed on the surface of the plates inoculated with the test microorganisms. The bacterial plates were incubated at 37°C for 18−24 hr, while the yeast plate was incubated at 30°C for 48 hr. Post-incubation, the zones of inhibition were measured and recorded. Usnic acid, identified as a bioactive metabolite in the lichen, was tested alongside the extracts. Vancomycin served as the positive control for *S. aureus* and MRSA, while gentamicin was used for the other bacteria and nystatin was used for *C. glabrata*. Paper discs loaded with methanol and chloroform served as negative controls.

Bioautography

TLC bioautography was used to determine the antibiotic compounds in lichen extracts. Briefly, each lichen extract was dissolved in chloroform (10 mg/mL) and loaded onto a TLC plate (silica gel) before being developed using an ethyl acetate-to-hexane ratio of 1:1 as the mobile phase. Then, the plate was transferred onto the culture medium that had been swabbed with the microbial indicator and incubated at 37°C for 24 hr. After incubation, the inhibitory zones were observed and the R_f values corresponding to these zones were recorded.

Purification and identification of bioactive compound

The bioactive compound in the lichen extract was purified using preparative TLC with an ethyl acetate-to-hexane ration of 1:1 as the mobile phase. The quenching band at the specified R_f value was scraped from the TLC plate and eluted using chloroform. Then, the eluted compound was evaporated to dryness, yielding a pale-yellow solid. The chemical structure of the purified compounds was identified using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR; Bruker Advance NEO 400 MHz NMR spectrometer; Karlsruhe, Germany), compared to the standard usnic acid (Sigma-Aldrich; Merck, Germany), as detailed in the Supplementary Data (Fig. S2-S6).

Results and Discussion

Preparation of lichen crude extracts

Crude extracts were obtained from each of the 14 lichen samples (PL01−PL14) using two different solvents (methanol or chloroform) to evaluate the extraction efficiency of each solvent. The yields of the crude extracts (expressed in milligrams per gram of dried lichen material) are visualized in Fig. 2.

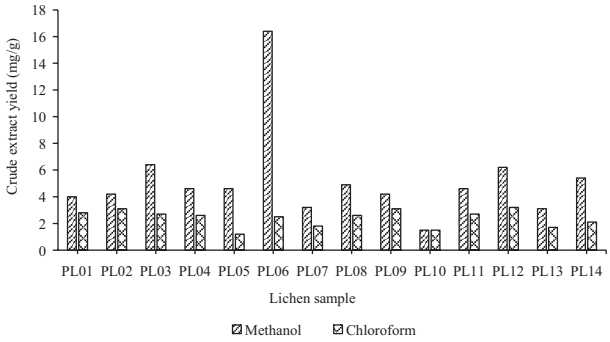


Fig. 2 Yields of crude extracts from lichen samples using methanol or chloroform as extraction solvents

Based on the extraction results, methanol generally provided higher yields than chloroform across most samples. For example, *Ramalina conduplicans* (PL01) yielded 4.0 mg/g when extracted with methanol, compared to 2.8 mg/g with chloroform. Similarly, *Parmotrema reticulatum* (PL02) had yields of 4.2 mg/g with methanol versus 3.1 mg/g with chloroform. The highest yield among the samples was from *Parmotrema tinctorum* (PL06) with 16.4 mg/g from methanol extraction. Based on these findings, methanol was generally more effective at solubilizing metabolites than chloroform in the majority of the cases examined. This variability in extract yield underscored the critical role of solvent selection in optimizing the extraction of secondary metabolites from lichens.

Various studies have explored different extraction methods for usnic acid from lichen. For example, Ahmad et al. (2017) demonstrated that ethanol was a more suitable solvent for extracting usnic acid from *Cladonia stellaris*, compared to acetone. Atila Dinçer et al. (2021) focused on optimizing the extraction using supercritical carbon dioxide, based on its efficiency in extracting active compounds without the use of toxic chemicals. Galanty et al. (2020) compared several techniques—heat reflux, shaking and ultrasound-assisted extractions—and found that heat reflux using acetone was the most effective method, potentially because of acetone's high solvency power at elevated temperatures which enhanced the extraction yield. Additionally, Cavalloro et al. (2021) used an innovative microwave-assisted extraction method involving ethanol, targeting (*S*)-usnic acid from *Cladonia foliacea*; they showed that rapid heating speeded up the extraction process while maintaining compound stability. These diverse studies highlighted that while traditional solvents like chloroform are effective, alternatives such as ethanol, supercritical carbon dioxide and acetone, could also be efficient in extraction, expanding the range of methods for extracting usnic acid from lichen sources.

Thin-layer chromatography analysis of lichens crude extract

The chemical profiles of the crude extracts from the lichen samples were investigated using TLC. The TLC analysis revealed distinct differences between the extraction capabilities of chloroform and methanol. Notably, only the chloroform extract from sample PL05 displayed a clear band corresponding to the usnic acid standard, indicating successful extraction of the usnic acid. The remaining chloroform extracts showed either smeared bands or bands located above the standard, perhaps suggesting the presence of impurities or other compounds. In contrast, the methanol extracts did not show any clear bands corresponding to usnic acid, with some samples only displaying smeared bands, further indicating the presence of impurities or a complex matrix that interfered with the clarity of the TLC results (Fig. 3). The successful extraction of usnic acid from sample PL05 (a species within the genus *Usnea*) aligned with the known biosynthetic capabilities of this genus to produce usnic acid. The presence of a distinct band in the chloroform extract of PL05 confirmed the solvent's suitability for extracting non-polar compounds, such as usnic acid, which correlated with the non-polar nature of chloroform (Kristmundsdóttir et al., 2005). The absence of similar results in the other samples suggested that either usnic acid was not present, the concentration was below detectable levels or the extraction was impeded by other co-extracted substances.

To further elucidate the solvent efficacy, the lichen samples PL11–PL14 (all in the *Usnea* genus) were subjected to extraction using both chloroform and methanol. Based on these results for TLC, the presence was confirmed of usnic acid in all extracts, with clear corresponding bands observed in both solvents (Fig. 4). Interestingly, while methanol effectively extracted usnic acid from sample PL12, it was unable to extract the compound from PL05, despite both being identified as *Usnea undulata*. This variation might be attributed to several factors, including biochemical diversity and differences in the physical structure among the lichens, environmental factors or variations in symbiotic relationships, which may have affected the concentration and accessibility of usnic acid. In addition, the chemical composition inside the lichen could potentially alter how successfully usnic acid dissolves in methanol.

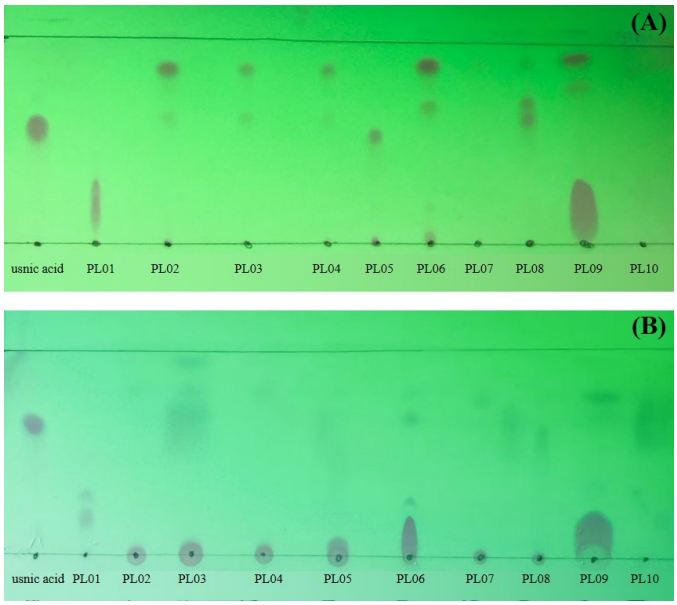


Fig. 3 Thin layer chromatography analysis of crude extracts from PL01–PL10 extracted using: (A) chloroform; (B) methanol

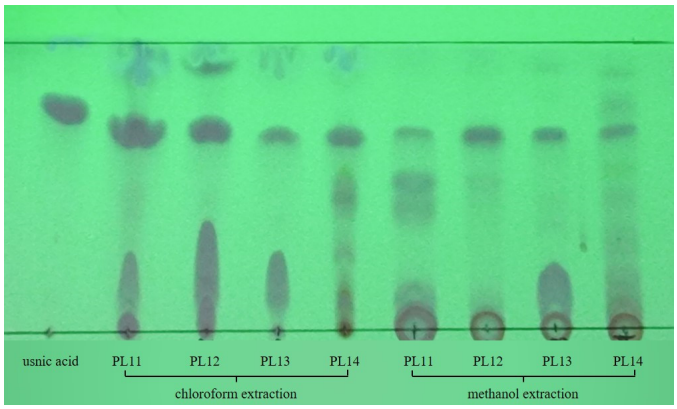


Fig. 4 Thin layer chromatography analysis of crude extracts from *Usnea* lichens PL11–PL14

Antimicrobial activity of crude extract

Based on the antimicrobial activity results from the crude extract using methanol and chloroform as solvents, it was clear that chloroform was a superior solvent for the extraction of antimicrobial compounds from lichens, as it consistently produced greater inhibition zones across the various tested pathogens than the methanol extraction. The antimicrobial assays conducted on the crude extracts from lichen samples PL01, PL05, PL08, PL09 and PL11–PL14 revealed varied inhibitory effects against a range of pathogenic microorganisms. Despite the absence of usnic acid in the TLC profiles of lichens PL01, PL08 and PL09, this observation suggested that other bioactive compounds were present within these extracts and had contributed to their antimicrobial properties. This corresponded with other studies that reported lichens as rich sources of unique bioactive compounds with potent antimicrobial properties. For example, lichens from the *Ramalina* genus are known to produce not only usnic acid but also other major secondary metabolites, such as salazinic acid and sekikaic acid, both of which have been shown to inhibit pathogenic bacteria (Kambar et al., 2014; Ankith et al., 2017). In addition, the antimicrobial properties of lichens from the genus *Parmotrema* have been explored extensively, particularly in *Parmotrema reticulatum*. Extracts from this lichen had major anti-inflammatory and antibacterial activities, making them effective against a variety of clinically relevant bacteria (Jain et al., 2016; Saha et al., 2021). Conversely, the lichen samples PL02, PL03, PL04, PL07 and PL10 had no inhibitory effects against the pathogenic microbes, indicating either a lack of antimicrobial compounds or an ineffective extraction method (Table 1).

Table 1 Antimicrobial activity against pathogenic microbes of crude extract from PL01–PL14

Sample	Extraction solvent	Inhibition zone diameter (mm)						
		B. subtilis	MRSA	*K. rhizophila*	*C. glabrata*	*S. aureus*	*S. mutans*	*S. epidermidis*
PL01	Methanol	16.2	0	26.28w	0	14.08	14.43	17.23
	Chloroform	24.55	0	29.17	0	14.45	0	17.45
PL02	Methanol	0	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0	0
PL03	Methanol	0	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0	0
PL04	Methanol	0	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0	0
PL05	Methanol	15.08	0	11.55	0	0	0	11.3
	Chloroform	24.05	0	27.08	0	11.05	9.05	18.1
PL06	Methanol	19.18	0	0	0	0	0	0
	Chloroform	0	11.18	0	0	0	0	0
PL07	Methanol	0	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0	0
PL08	Methanol	22.25	12.33	24.17w	0	10.78	12.03	18.53
	Chloroform	24.30	14.28	34.17w	0	12.10	12.68	21.2
PL09	Methanol	19.90	10.08	21.10w	0	10.10	10.63	9.05
	Chloroform	16.15	10.10	25.08	0	11.80	11.48	11.73
PL10	Methanol	0	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0	0
PL11	Methanol	13.33	0	11.60	0	0	7.78	23.45
	Chloroform	26.15	12.55	29.83	0	12.10	11.10	29.73
PL12	Methanol	21.18	9.85	29.88	20.15w	10.70	10.38	27.40
	Chloroform	26.53	12.65	29.90	0	13.30	12.63	31.63

Table 1 Continued

Sample	Extraction solvent	Inhibition zone diameter (mm)						
		B. subtilis	MRSA	*K. rhizophila*	*C. glabrata*	*S. aureus*	*S. mutans*	*S. epidermidis*
PL13	Methanol	19	9.03	19.70	0	9.60	9.28	25.18
	Chloroform	22.08	11.58	25.95	0	11.80	11.15	29.03
PL14	Methanol	16.23	18.95	23.63	0	10.10	10.23	26.33
	Chloroform	22.13	14.43	27.60	0	12.50	11.10	28.13
Usnic acid	-	17.85	7.70	20.30	0	7.20	7.15	21.15
Gentamycin	-	20	NA	29.70	NA	NA	21.25	29.20
Vancomycin	-	NA	18.95	NA	NA	18.45	NA	NA
Nystatin	-	NA	NA	NA	22.25	NA	NA	NA

MRSA = Methicillin-resistant *Staphylococcus aureus*; w = weak inhibition zone; NA = not applicable.

The increase in drug-resistant bacteria presents a major challenge to global public health organizations. MRSA is a type of bacteria that has developed resistance to many antibiotics, including methicillin and other common antibiotics such as penicillin, amoxicillin and oxacillin (Batabyal et al., 2012). Although *S. aureus* is mostly harmless on human skin, it can lead to severe infections if it infiltrates the body through a laceration, wound, catheter or respiratory tube (Alebachew et al., 2012; Prince, 2013; Yedikian et al., 2023). MRSA demonstrates antibiotic resistance through various mechanisms, with the main one including the mecA gene, which produces a mutated penicillin-binding protein (PBP2a) that has a reduced ability to bind to beta-lactam antibiotics (Lade et al., 2022). In addition, MRSA uses efflux pumps to flush out antibiotics, creates protective biofilms and obtains resistance genes through horizontal gene transfer (Piechota et al., 2018; Vestergaard et al., 2019).

The bio-efficacy of usnic acid has been extensively demonstrated in numerous studies, particularly regarding its antimicrobial and cytotoxic properties. The antimicrobial potential has been well-documented, as shown by Tay et al. (2004), who reported inhibitory activity against various microorganisms, including *S. aureus*, with minimum inhibitory concentration (MIC) values as 3.1 µg/62.5 µL. In the present study, usnic acid had a strong inhibitory effect against MRSA, further emphasizing its potency against drug-resistant bacterial strains. Similarly, Ranković et al. (2012) reported on the broad-spectrum antimicrobial efficacy of usnic acid, with MIC values in the range 0.0008–0.5 mg/mL, confirming its effectiveness across various bacterial species. Additionally, Zuo et al. (2018) demonstrated that usnic acid was highly effective against MRSA, underscoring its potential as a key compound in the treatment of resistant infections. In terms of its mechanism of action, usnic acid is known to disrupt bacterial membranes, thereby reducing cell viability without damaging the cell wall, with this disruption enhancing the efficacy of antibiotics when used in combination, exhibiting synergistic effects that increase antibacterial potency (Segatore et al., 2012; Kıvanç et al., 2023). Furthermore, usnic acid has been shown to inhibit MRSA biofilm formation through multiple pathways. For example, Pompilio et al. (2016) found that usnic acid impaired the adhesion of bacteria to host matrix binding proteins, while simultaneously decreasing the expression of enzymes crucial for biofilm development. Additionally, Sinha et al. (2019) reported that usnic acid down-regulated the proteins involved in peptidoglycan and fatty acid synthesis, further inhibiting bacterial growth and biofilm formation.

Furthermore, based on the present results, even crude extracts with undetectable levels of usnic acid, as evidenced by the TLC results, demonstrated potent antimicrobial activity against a broad spectrum of pathogenic bacteria, including drug-resistant strains such as MRSA. Notably, the crude extracts from the samples coded PL08 and PL09 (containing no detected usnic acid) were effective in inhibiting MRSA. This suggested that compounds other than usnic acid in the lichen extracts had substantial antimicrobial properties. These results underscored the potential of lichen-derived compounds as promising candidates for developing new antimicrobial agents, particularly in the fight against drug-resistant bacteria. Further studies are warranted to isolate and characterize these additional bioactive compounds to fully understand their mechanisms of action and therapeutic potential. Among the pathogens tested, *K. rhizophila* was the most susceptible, displaying the greatest average inhibition zones. This suggested the higher sensitivity of *K. rhizophila* to the antimicrobial compounds present in the lichen extracts, which could be attributed to the specific interaction between usnic acid and the cellular components of *K. rhizophila*. Although usnic acid has been reported to exhibit inhibitory effects against various bacteria (Kambar et al., 2014; Maulidiyah et al., 2020; Kıvanç et al., 2023), its effect on *K. rhizophila* has not been directly reported. Therefore, *K. rhizophila* was selected for the bioautography tests.

The chloroform-derived crude extract was utilized in these assays due to its superior inhibitory efficacy observed across multiple microbial species. The results demonstrated that all extracts produced clear inhibition zones against *K. rhizophila*, indicating antimicrobial activity. In particular, the extracts from PL11–PL14 had R_f values and diameter of inhibition zones somewhat similar to usnic acid, suggesting the presence of compounds with potent antimicrobial properties (Fig. S7).

Purification and identification of usnic acid from purified crude extract

Initially, the lichen crude extracts were purified using preparative TLC. During this process, the quenching bands observed were scraped from the TLC plate and eluted using chloroform. The purified crude extracts were subjected to a second TLC analysis. These results revealed that the purified extracts from the chloroform extractions of samples PL11–PL14 each displayed a single band that corresponded with the standard usnic acid band (Fig. S8). These purified compounds were subjected to NMR analysis to further confirm their identity.

The purified compounds PL11–PL14 appeared as pale-yellow solids. To verify their chemical structures, (+)-usnic acid was used as a standard and analyzed using ^{1}H-NMR. Then, the NMR spectra of the standard were compared with those of compounds PL11–PL14. The ^{1}H-NMR data of isolated usnic acid presented as: ^{1}H NMR (400 MHz, $CDCl_3$): PL11 δ 1.76, 2.11, 2.66, 2.68, 5.98, 7.26, 11.03, 13.32, 18.84; PL12 δ 1.76, 2.11, 2.66, 2.68, 5.98, 7.26, 11.03, 13.32, 18.84; PL13 δ 1.76, 2.11, 2.66, 2.68, 5.98, 7.26, 11.03, 13.32, 18.84; PL14 δ 1.76, 2.11, 2.66, 2.68. 5.98, 7.26, 11.03, 13.32, 18.84. The ^{1}H-NMR spectra of all compounds resembled that of the usnic acid standard. Therefore, it was concluded that compounds PL11–PL14 were usnic acid (Fig. S6).

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This project was supported by the National Research Council of Thailand. The Faculty of Pharmaceutical Sciences, Chulalongkorn University provided their research facilities.

References

Ahmad, R., Borowiec, P., Falck-Ytter, A.B., Strætkvern, K.O. 2017. Extraction, solubility and antimicrobial activity of (-) usnic acid in ethanol, a pharmaceutically relevant solvent. Nat. Prod. Commun. 12: 1101−1104. doi.org/10.1177/1934578x1701200725

Alebachew, T.A., Yismaw, G., Derabe, A., Sisay, Z. 2012. *Staphylococcus aureus* burn wound infection among patients attending Yekatit 12 Hospital Burn Unit, Addis Ababa, Ethiopia. Ethiop. J. Health Sci. 22: 209−213.

Ankith, G., Kekuda, T.R.P., Rajesh, M.R., Karthik, K.N., Avinash, H.C., Vinayaka, K.S. 2017. Antibacterial and antifungal activity of three *Ramalina* species. J. Drug Deliv. Ther. 7: 27−32. doi.org/10.22270/jddt.v7i5.1501

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., et al. (2021). Natural products in drug discovery: advances and opportunities. Nat. Rev. Drug Discovery. 20(3): 200–216. doi.org/10.1038/s41573-020-00114-z

Dinçer, C.A., GÖKalp, C., Getiren, B., Yildiz, A., Yildiz, N. 2021. Supercritical carbon dioxide extraction of *Usnea longissima* (L.) Ach.: Optimization by Box-Behnken design (BBD). Turk. J. Chem. 45: 1248−1256. doi.org/10.3906/kim-2102-66

Batabyal, B., Biswas, S., Bengal, W. (2012). Methicillin-Resistant *Staphylococcus aureus*: A Brief Review. Int. Res. J. Biological Sci. 1: 65-71.

Buaruang, K., Boonpragob, K., Mongkolsuk, P., Sangvichien, E., Vongshewarat, K., Polyiam, W., Rangsiruji, A., Saipunkaew, W., et al. 2017. A new checklist of lichenized fungi occurring in Thailand. MycoKey. 23: 1-91. doi.org/10.3897/mycokeys.23.12666

Cavalloro, V., Marrubini, G., Stabile, R., Rossi, D., Linciano, P., Gheza, G., Assini, S., Martino, E., Collina, S. 2021. Microwave-assisted extraction and HPLC-UV-CD determination of (S)-usnic acid in *Cladonia foliacea*. Molecules. 26: 455. doi.org/10.3390/molecules26020455

Galanty, A., Pasko, P., Podolak, I., Zagrodzki, P. 2020. Optimization of usnic acid extraction conditions using fractional factorial design. The Lichenologist. 52: 397-401. doi.org/10.1017/S0024282920000316

Ingólfsdóttir, K. (2002). Usnic acid. Phytochemistry. 61(7): 729–736. doi.org/10.1016/s0031-9422(02)00383-7

Ivanova, D., Ivanov, D. 2009. Ethnobotanical use of lichens: Lichens for food review. Scr. sci. med. 41: 11. doi.org/10.14748/ssm.v41i1.456

Jain, A.P., Bhandarkar, S., Rai, G., Yadav, A.K., Lodhi, S. 2016. Evaluation of *Parmotrema reticulatum* Taylor for antibacterial and anti-inflammatory activities. Indian J. Pharm. Sci. 78: 94−102. doi.org/10.4103/0250-474x.180241

Kambar, Y., Vivek, M.N., Manasa, M., Kekuda, T.R.P., Onkarappa, R. 2014. Antimicrobial activity of *Ramalina conduplicans* Vain. (*Ramalinaceae*). Sci. Technol. Arts Res. 3: 57-62. doi.org/10.4314/star.v3i3.10Kıvanç, S.A., Akova, B., Kıvanç, M. 2023. Effects of the dibenzofuran, usnic acid, on inhibition of ocular biofilm formation due to coagulase-negative *Staphylococci*. Med. Sci. Monit. 29: e940266. doi.org/10.12659/msm.940266

Kristmundsdóttir, T., Jónsdóttir, E., Ögmundsdóttir, H.M., Ingólfsdóttir, K. 2005. Solubilization of poorly soluble lichen metabolites for biological testing on cell lines. Eur. J. Pharm. Sci. 24: 539-543.

Lade, H., Joo, H.S., Kim, J.S. 2022. Molecular basis of non-β-lactam antibiotics resistance in *Staphylococcus aureus*. Antibiotics. 11: 1378. doi.org/10.3390/antibiotics11101378

Maulidiyah, M., Darmawan, A., Erviana, A., Andi, M., Wibowo, D., Salim, L.O.A., Arham, Z., Mustapa, F., Adiba, N., Nurdin, M. 2020. Antioxidant activity-guided isolation of usnic acid and diffractaic acid compounds from lichen genus *Usnea* sp. J. Appl. Pharm. Sci. 11: 75-83. doi.org/10.7324/JAPS.2021.110210

Piechota, M., Kot, B., Frankowska-Maciejewska, A., Grużewska, A., Woźniak-Kosek, A. 2018. Biofilm formation by methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains from hospitalized patients in Poland. Biomed. Res. Int. 2018: 4657396. doi.org/10.1155/2018/4657396

Pompilio, A., Riviello, A., Crocetta, V., Giuseppe, F.D., Pomponio, S., Sulpizio, M., Ilio, C.D, Angelucci, S., Barone, L., Giulio, A.D, Bonaventura, G.D. 2016. Evaluation of antibacterial and antibiofilm mechanisms by usnic acid against methicillin-resistant *Staphylococcus aureus*. Future Microbiol. 11: 1315−1338. doi.org/10.2217/fmb-2016-0049

Prince, A. 2013. *Staphylococcus aureus* Infection in the respiratory tract. In: Prince, A. (Ed.). *Mucosal Immunology of Acute Bacterial Pneumonia*. Springer. New York, NY, USA. pp. 239−258.

Ranković, B., Kosanić, M., Stanojković, T., Vasiljević, P., Manojlović, N. 2012. Biological activities of *Toninia candida* and *Usnea barbata* together with their norstictic acid and usnic acid constituents. Int. J. Mol. Sci.13: 14707−14722. doi.org/10.3390/ijms131114707

Rassabina, A., Khabibrakhmanova, V., Babaev, V., Daminova, A., Minibayeva, F. 2022. Melanins from the lichens *Lobaria pulmonaria* and *Lobaria retigera* as eco-friendly adsorbents of synthetic dyes. Int. *J. Mol. Sc*i. 23: 15605. doi.org/10.3390/ijms232415605

Ren, M., Jiang, S., Wang, Y., Pan, X., Pan, F., Wei, X. 2023. Discovery and excavation of lichen bioactive natural products. Front Microbiol. 14: 1177123. doi.org/10.3389/fmicb.2023.1177123

Saha, S., Pal, A., Paul, S. 2021. A review on pharmacological, anti-oxidant activities and phytochemical constituents of a novel lichen *Parmotrema* species. J. Biol. Active Prod. Nature. 11: 190−203. doi.org/10.1080/22311866.2021.1916596

Segatore, B., Bellio, P., Setacci, D., Brisdelli, F., Piovano, M., Garbarino, J.A., Nicoletti, M., Amicosante, G., Perilli, M., Celenza, G. 2012. In vitro interaction of usnic acid in combination with antimicrobial agents against methicillin-resistant *Staphylococcus aureus* clinical isolates determined by FICI and ΔE model methods. Phytomedicine. 19: 341−347. doi.org/10.1016/j.phymed.2011.10.012

Sinha, S., Gupta, V.K., Kumar, P., Kumar, R., Joshi, R., Pal, A., Darokar, M.P. 2019. Usnic acid modifies MRSA drug resistance through down-regulation of proteins involved in peptidoglycan and fatty acid biosynthesis. FEBS Open Bio. 9: 2025−2040. doi.org/10.1002/2211-5463.12650

Tagirdzhanova, G., Saary, P., Cameron, E.S., Garber, A.I., Escandón, D.D., Goyette, S., Nogerius, V.T., Passo, A., Mayrhofer, H., Holien, H., Tønsberg, T., Stein, L.Y., Finn, R.D., Spribille, T. 2023. Evidence for a core set of microbial lichen symbionts from a global survey of metagenomes. bioRxiv. 2023.2002.2002.524463. doi.org/10.1101/2023.02.02.524463

Tay, T., Türk, A. Ö., Yılmaz, M., Türk, H., Kıvanç, M. 2004. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstictic acid, and protocetraric acid constituents. Z. Naturforsch. C. 59: 384−388.

Vestergaard, M., Frees, D., Ingmer, H. 2019. Antibiotic resistance and the MRSA problem. Microbiol Spectr. 7: 10.1128/microbiolspec.gpp1123-0057-2018. doi.org/10.1128/microbiolspec.gpp3-0057-2018

Yedikian, T., Lescott, C., Rego, E., Girgis, K., Javed, S., Elsayed, D., Sodha, A. 2023. Methicillin-resistant *Staphylococcus aureus* lip abscess as a complication of facial laceration. Am. J. Med. Case Rep. 11: 116−118. doi.org/10.12691/ajmcr-11-7-1

Zuo, G.Y., Fu, R.C., Yu, W., Zhang, Y.L., Wang, G.C. 2018. Potentiation effects by usnic acid in combination with antibiotics on clinical multi-drug resistant isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Med. Chem. Res. 27. doi.org/10.1007/s00044-018-2161-5