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Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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To cite this article: Ari Satia Nugraha, Ludmilla Fitri Untari, Annegret Laub, Andrea Porzel, Katrin Franke & Ludger A. Wessjohann (2020): Anthelmintic and antimicrobial activities of three new depsides and ten known depsides and phenols from Indonesian lichen: *Parmelia cetrata* Ach., Natural Product Research, DOI: 10.1080/14786419.2020.1761361

To link to this article: https://doi.org/10.1080/14786419.2020.1761361

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1761361

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Anthelmintic and antimicrobial activities of three new depsides and ten known depsides and phenols from Indonesian lichen: *Parmelia cetrata* Ach.

Ari Satia Nugraha[a,b], Ludmilla Fitri Untari[c], Annegret Laub[b], Andrea Porzel[b], Katrin Franke[b] and Ludger A. Wessjohann[b]

[a]Drug Utilisation and Discovery Research Group, Faculty of Pharmacy, University of Jember, Jember, Indonesia; [b]Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany; [c]Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT

An extensive phytochemical study of a foliose lichen from Indonesia, *Parmelia cetrata*, resulted in the successful isolation of 13 phenol and depside derivatives (**1**–**13**) including the previously unreported depsides 3′-hydroxyl-5′-pentylphenyl 2,4-dihydroxyl-6-methylbenzoate (**7**), 3′-hydroxyl-5′-propylphenyl 2,4-dihydroxyl-6-methylbenzoate (**8**) and 3′-hydroxyl-5′-methylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**9**). The anti-infective activity of isolated compounds was evaluated against the gram-negative bacterium *Aliivibrio fischeri* and the nematode *Caenorhabditis elegans*. 2,4-Dihydroxyl-6-pentylbenzoate (**5**) and lecanoric acid (**6**) induced growth inhibition of *A. fischeri* with inhibition values of 49% and 100% at a concentration of 100 μM, respectively. The antibacterial activity might be due to their free carboxyl group. A phenolic group at C4 also contributed to the antimicrobial activity of the depsides as shown for compounds **7** and **8**, which caused 89% and 96% growth inhibition at 100 μM, respectively. Lecanoric acid (**6**) in addition possesses significant anthelmintic effects causing 80% mortality of *C. elegans* at 100 μg/mL.

ARTICLE HISTORY
Received 4 March 2020
Accepted 22 April 2020

KEYWORDS
Lichen; *Parmelia cetrata*; antimicrobial; anthelmintic; depsides; *Aliivibrio fischeri*; *Caenorhabditis elegans*

1. Introduction

Lichen is a unique biomass composed of a fungus in symbiosis with algae, cyanobacteria or non-photosynthetic bacteria which behave as cosmopolitan species growing

CONTACT Ludger A. Wessjohann wessjohann@ipb-halle.de; Ari Satia Nugraha arisatia@unej.ac.id
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1761361.

from dry cold pole to warm and humid tropic regions (Brodo et al. 2001; Selbmann et al. 2010). More than 100,000 lichen species have been reported, of which 17,000 species grow in Indonesia (Negi 2003). Over 1050 secondary metabolites have been isolated from lichens and characterized as depsides, depsidones, xanthones and terpenes which possess antibacterial, antiviral, anti-analgesic, antipyretic and antiproliferative activities (Müller 2001; Molnár and Farkas 2010).

An antibacterial activity of lichens was first reported in 1944. The study on 42 lichen species indicated 27 crude extracts to possess significant activity against gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* (Burkholder et al. 1944; Shrestha and St Clair 2013). Meanwhile, thirty-four lichen species of North America were screened against the gram-negative bacteria *Pseudomonas aeruginosa*, of which 16 species indicated promising activities with MIC values of $<16\,\mu g/mL$ (Shrestha et al. 2014). The antimicrobial activity of metabolites from Chilean lichens was previously evaluated against methicillin-resistant *Staphylococci*. The most active depside sphaerophorin possessed a MIC value of $8\,\mu g/mL$ (Celenza et al. 2013).

The archipelagic country of Indonesia has the second largest biodiversity in the world and is composed by Australian and Asian species realms, covering 17,000 islands, from coastal humid vegetation to temperate mountainous vegetation (Nugraha and Keller 2011). Scientific exploration on Indonesian biodiversity is progressing yet limited, including the Indonesian lichens. The earliest study record on Indonesian lichen was published by Merrill in 1913 in which samples were collected by Mr. Fleischer from various locations on Java island (Merrill 1913). The study merely reported for botanical and taxonomical purposes. Due to their low biomass, only very few lichen genera have been part of traditional medication of the indigenous people of Indonesia, including the genus *Usnea*. The decocted plant was traditionally prescribed to treat diarrhoea, dysentery, aphthous ulcers, abdominal distention and inflammation. *Usnea misaminensis* possesses anti-infective activity against *Mycobacterium tuberculosis* and *Plasmodium falciparum* due to the content of usnic acid and salazinic acid (Nugraha, Wangchuk, et al. 2019). Our previous preliminary screening on nine foliose lichens from Indonesia indicated that the crude methanol extracts possess anti-microbial and anticancer activities with *Parmelia cetrata* being the most effective one (Nugraha, Pratoko, et al. 2019). In the current research a detailed phytochemical and pharmacological evaluation of *P. cetrata* from Java was performed. The anti-infective potency of isolated constituents was evaluated based on antimicrobial activity tests against the gram-negative model bacterium *Aliivibrio fischeri* and anthelmintic activity tests against the helminth model *Caenorhabditis elegans*.

2. Result and discussion

2.1. Isolation and structure elucidation of constituents of Parmelia cetrata

Parmelia cetrata Ach. (Figure S26) is a foliose lichen with light grey lobate lobes, irregular, upper cortex reticulately cracked to the margin, rhizines margin with black cilia, lacking of soredia and isidia; lower surface black with a narrow brown marginal zone, black rhizines dense below, medulla K + yellow. Apothecia were not found on the specimen studied. *Parmelia acanthifolia* Pers., *Parmelia cetrata* Ach., *Parmelia herrei*

Zahlbr., *Parmelia reparata* Stirt. *Parmotrema cetratum* (Ach.) Hale and *Rimelia cetrata* (Ach.) Hale & A. Fletcher are considered the taxonomic synonyms of *P. cetrata, sometimes also misspelled Pharmelia cetrata/citrata*. The species distribution of *P. cetrata* is cosmopolitan with wide spreading on deciduous trees in open woods. It could be found in tropical forests in lowland to sub-montane areas. However, since this species has never been subjected to any taxonomic studies in Indonesia, species distribution data in Indonesia are lacking. Our preliminary bioprospecting studies on lichens of Java island revealed the *P. cetrata* crude methanol extract to possess activity against the human pathogenic gram-negative bacterium *Pseudomonas aeruginosa* in which 1024 μg/mL concentration produced 50% bacterial inhibition (Nugraha, Pratoko, et al. 2019).

Although the lichen belongs to the foliose lichen group, obtaining reasonable amounts for bioactivity and isolation studies was challenging. Therefore, the fractions obtained from the crude methanol extract were directly profiled by analytical HPLC prior to preparative HPLC application. All the samples were cleaned through a C_{18} solid phase extraction (SPE) medium. Extensive chromatographic work resulted in the isolation of 13 compounds (Figure 1) comprising ten known compounds, 5-methylresorcinol (**1**) (Ivanova et al. 2010), 5-propylresorcinol (**2**) (Schmeda-Hirschmann et al. 2008), methyl 2,4-dihydroxy-6-methylbenzoate (**3**) (Ango et al. 2016), methyl 2-hydroxy-4,6-dimethylbenzoate (**4**) (Chan and Brownbridge 1980), 2,4-dihydroxy-6-pentylbenzoate (**5**) (Lin et al. 2013), lecanoric acid (**6**) (Ivanova et al. 2010), 3′-hydroxy-5′-propylphenyl 2-hydroxy-4-methoxy-6-propylbenzoate (**10**), 3′-hydroxy-5′-pentylphenyl 2-hydroxy-4-methoxy-6-propylbenzoate (**11**), 3′-hydroxy-5′-propylphenyl 2-hydroxy-4-methoxy-6-pentylbenzoate (**12**), 3′-methoxy-5′-propylphenyl 2-hydroxy-4-methoxy-6-propylbenzoate (**13**) (Elix and Wardlaw 1997) and the three new depsides **7**–**9**. Their molecular structures were unequivocally established based on spectroscopic and spectrometric spectral data analysis and on comparison to literature data cited above. NMR spectral data analyses of the previously undescribed compounds **7**–**9** are summarised in Table S24. To the best of our knowledge, these depsides are reported for the first time (Figure 1).

Although naturally rare, alkyl resorcinols **1**–**5** are part of phenolic lipids which are well distributed in vascular plants, mosses, mushrooms, and fungi. Plants use the orcinols to protect against hostile fungi and bacteria (Vagel and Roo 2004). The molecular structures of **6**–**13** represent depsides which are typical secondary metabolites, produced by lichens especially from Parmeliaceae family (Gianini et al. 2008).

Compound **7** was isolated from the *n*-hexane fraction as pale brown amorphous solid with UV absorbing activity. The IR spectrum indicates vibrational bands at 3336 and 1587 cm^{-1}, assigned to hydroxyl and ester carbonyl stretches, respectively. The molecular formula $C_{19}H_{22}O_5$ of compound **7** was confirmed by HRESIMS with an assigned $[M-H]^-$ peak at *m/z* 329.1391. NMR data (Table S24) are in accordance with a depside characteristic for lichens. A sharp peak at δ 11.34 ppm in the ^{1}H-NMR spectrum is evident for a typical phenol-carbonyl hydrogen bond of *ortho*-hydroxyl benzoates. Two doublet protons ($J = 2.4$ Hz) at δ 6.30 and 6.38 ppm represent *meta* coupled protons of H3 and H5. These two protons exhibit a long-range correlation with a carbonyl group at δ 171.0 ppm (C = O) based on gHMBC spectral analysis

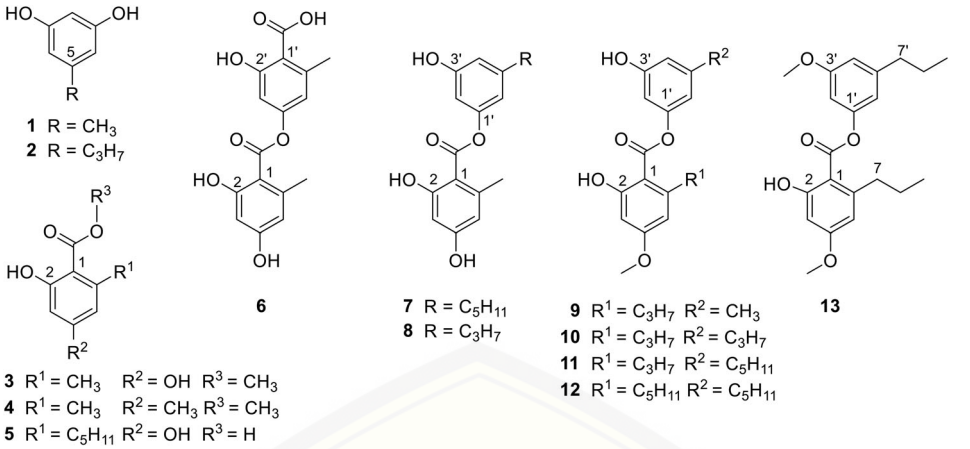

Figure 1. Phenols (**1**–**5**) and depsides (**6**–**13**) isolated from the foliose lichen *Parmelia cetrata*.

(Figure S5). A singlet signal at δ 2.60 ppm indicates an aromatic methyl group which correlated to the carbonyl group at δ 171.0 ppm. ^{1}H-NMR and gCOSY spectra show three doublet of doublet peaks ($J = 2.0$, 2.0) at δ 6.60 (H2′), 6.63 (H6′), 6.66 (H4′), clearly indicating an 1,3,5-trisubstituted benzene. Furthermore, ^{1}H-NMR and gTOCSY spectra displayed a proton peak system at δ 0.90 (dd, $J = 7.2$, 7.2, 3H, H, H11′), 1.35 (m, 4H, H10′ and H9′), 1.63 (2H, H8′) and 2.58 ppm (dd, $J = 7.8$, 7.8 Hz, 2H, H7′) evident for an aliphatic chain (-C_5H_{11}). The gHMBC analysis proved the attachment of this alkyl to C5′. HMBC correlations are summarised in Figure S27. Overall, these analyses suggest for compound **7** the depside structure of 3′-hydroxyl-5′-pentylphenyl 2,4-dihydroxyl-6-methylbenzoate.

Compound **8** was isolated from the *n*-hexane fraction as a pale brown amorphous solid with UV absorbing activity. The IR spectrum indicated similarity to compound **7** with hydroxyl (3345 cm^{-1}) and carbonyl stretches (1656 cm^{-1}). The molecular formula $C_{17}H_{18}O_5$ of compound **7** was confirmed by HRESIMS with an assigned $[M-H]^-$ peak at *m/z* 301.1079. The ^{1}H-NMR and gHSQC spectra showed similar pattern like compound **7** with a clear difference. In compound **8** a shorter aliphatic chain, a propyl group, is attached to C6′. Compound **8** was identified as 3′-hydroxyl-5′-propylphenyl 2,4-dihydroxyl-6-methylbenzoate.

Compound **9** was isolated as an UV active, brown amorphous solid. The IR spectrum indicated again stretches at 3307 and 1615 cm^{-1}, assigned to hydroxyl and carbonyl stretches, respectively. The molecular formula $C_{18}H_{20}O_5$ was deduced from its $[M-H]^-$ peak at *m/z* 315.1237 in HRESIMS. The ^{1}H-NMR and gHSQC spectra indicated similarities to compounds **7** and **8**, however, the substituents at position 6′ and 2 are exchanged. The aliphatic chain attached to C6′ is replaced by a methyl group in **9**, and a propyl group is attached to C2 instead of the methyl group in **7** and **8**. In addition, another distinct proton signal at δ 3.86 ppm suggests the presence of a methoxyl group. Long range correlations from these protons to C4 indicate the location of the methoxyl group at position C4. Compound **9** was identified as 3′-hydroxyl-5′-methylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate.

2.2. Antimicrobial and anthelmintic activities of compounds 1–13

To evaluate the anti-infective properties, the isolated compounds were tested against the gram-negative bacterium *A. fischeri* and against the helminth *C. elegans*. Both are non-pathogenic model test organism which can be used for the initial screening of antibacterial or anthelmintic compounds.

The relative antimicrobial activities of resorcinols and depsides against *A. fischeri* are shown in Figure S28 and are summarised in Table S25. Lecanoric acid (**6**) possesses the highest antibacterial activity, completely preventing the growth of *A. fischeri* at a concentration of 100 μM (Figure S27). Free benzoic acid groups are commonly accepted to be responsible for antimicrobial activity as also shown for compound **5** (Cho et al. 1998). Esterification of this group, as present in compounds **3** and **4**, significantly relegate the antimicrobial activity. Moreover, absence of a free acid group decreased the antimicrobial activity as shown for compounds **1**, **2**, **7**–**13**. However, the occurrence of a phenolic group at C4 significantly elevated the antibacterial activity against the gram-negative test organism as observed in compounds **7** and **8** with inhibition rates above 80%. Comparing the two, the more lipophilic compound **7** fares better. Overall, the combination of typical antimicrobial descriptors of depsides, a free carboxylic acid group along with a phenolic group at C4, exists in lecanoric acid (**6**).

In the anthelmintic bioassay, lecanoric acid (**6**) possessed the highest activity among the despside derivatives, although it is 20% lower than the maximum response of the positive control (ivermectin) (Figure S28). The remaining depsides were considered as inactive causing helminth mortalities under 50%.

Lecanoric acid (**6**) was previously reported to be active against several pathogenic bacteria and fungi, including *Bacillus mycoides*, *Bacillus subtilis*, *Enterobacter cloaceae*, *Eschericia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Candida albicans*, *Fusarium oxysporum*, *Mucor mucedo*, *Paecilomyces variotii*, *Penicillium purpurescens*, *Penicillium verrucosum* and *Trichoderma harsianum* with MIC values between 0.062 and 0.125 μg/mL (Hew and Gam 2010). Previous pharmacological studies revealed compound **3** to inhibit bacterial and fungal growth of *Providencia stuartii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Candida albicans* with considerable higher MIC values of 256, 512, 256, 128, 256, 512, 256 and 512 μg/mL, respectively (Ango et al. 2016). There were also previous pharmacological studies on the known depsides and decarboxylated didepsides **10**–**13** (Elix and Wardlaw 1997).

3. Experimental

3.1. General experimental procedures

UV–visible spectra of samples diluted in MeOH were obtained using a Jasco V-560 UV/Vis spectrophotometer. IR spectra were recorded with a Thermo Nicolet 5700 FT-IR spectrometer. 1D (1H, 13C) and 2D (gCOSY, TOCSY, ROESY, gHSQC, gHMBC) NMR spectra were recorded from an Agilent VNMRS 600 system. The ^{1}H and ^{13}C NMR spectra were measured at 600 MHz and 150 MHz, respectively. Chemical shifts were referenced to internal TMS (δH 0, ^{1}H), or solvent signals $(CD_3)_2CO$ (δC 29.8, ^{13}C) or CD_3OD (δC

49.0, ^{13}C). The mixing time for the TOCSY and ROESY experiments were set to 0.4 s. The negative ion high resolution ESI mass spectra were obtained from an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with an HESI electrospray ion source. The data were evaluated by the Xcalibur software 2.7 SP1. Analytical HPLC was performed on a Shimadzu HPLC Prominence system (DGU-20ASR degassing unit, LC-20AT pump, SIL-20AHT auto sampler, SPD-M20A diode array detector, CBM-20A communications bus module, controlled by Shimadzu LabSolution software) with a Symmetry C_{18} column (5 μm, 4.9 × 150 mm). Preparative HPLC was performed on a Knauer prep-LC system (WellChrom pump K-1001, WellChrom UV detector K-250-1, Knauer dynamix mixing chamber, Rheodyne 7725i injector, controlled by EuroChrom 2000 V3.05 software) with a YMC J'sphere M-80 column (4 μm, 10 × 250 mm). Absorbance for antimicrobial assays was measured on a Spark Tecan microplate reader. *Caenorhabditis elegans* morbidity was observed under an Olympus BX41 microscope (Model BX41TF) with 40-fold magnification. All solvents were HPLC grade bought from Merck. Ivermectin standard and penicillin-streptomycine solution were obtained from Sigma-Aldrich

3.2. Lichen material

The lichen sample was collected from Sukosari, Bondowoso Regency, Indonesia (N-7.969496°, E114.025594°) at 1500 metres above sea level. Fresh lichen biomass (240 g) was collected from three *Swietenia mahogany* trees in the same location. The species was determined as *Parmelia cetrata* Ach. by Ludmilla Fitri Untari, a lichenologist at Laboratory of Botany, Faculty of Biology, Universitas Gadjah Mada, Indonesia where the voucher sample is deposited under the code LCB01. *Parmelia cetrata* Ach. is currently considered as synonym of *Rimelia cetrata* (Arch.) Hale & Fletcher and *Parmotrema cetratum* (Ach.) Hale.

3.3. Extraction and isolation

The lichen biomass was cleaned, dried and ground with liquid nitrogen assistance. Lichen powder (150 g) was loaded into an Erlenmeyer flask (500 mL), soaked with methanol (400 mL) and stirred for 24 h. The supernatant was collected and vacuum dried to produce the crude methanol extract (9.9 g). A portion of crude extract (2.5 g) was redissolved in methanol:water (1:9; 100 mL) and was sequentially extracted with *n*-hexane (15 × 100 mL) and ethyl acetate (15 × 100 mL) followed by vacuum drying to produce the *n*-hexane fraction (100 mg) and ethyl acetate fraction (1.99 mg).

The dried *n*-hexane fraction (100 mg) was loaded into an Isolute^{TM} SPE column (C_{18}, 5 g, 20 mL) connected with a 0.45 μm HPLC sample filter. The solution was then deployed into a semi-preparative HPLC with a gradient from 2% to 50% solvent B in 2 min, 50% to 55% solvent B in 18 min, 55% to 100% solvent B in 10 min (solvent A: water; solvent B: acetonitrile, flow rate 3 mL/min). The compounds 5-propylresorcinol (**2**, 1.7 mg), methyl 2,4-dihydroxyl-6-methylbenzoate (**3**, 5.0 mg), methyl 2-hydroxyl-4,6-dimethylbenzoate (**4**, 3.2 mg), 2,4-dihydroxyl-6-pentylbenzoate (**5**, 5.4 mg), 3′-hydroxyl-5′-methylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**9**, 5.2 mg), 3′-hydroxyl-5′-

propylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**10**, 3.4 mg), 3′-hydroxyl-5′-pentylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**11**, 1.0 mg), 3′-methoxyl-5′-propylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**13**, 1.0 mg), were collected at t_R 7.7, 8.5, 9.3, 12.4, 14.3, 20.1, 26.6 and 28.4 min, respectively.

Part of the ethyl acetate fraction (500 mg) was loaded into an Isolute^{TM} SPE column ($C_{18,}$ 5 g, 20 mL) and subsequently eluted with 50% methanol in water (10 mL), 75% methanol in water (20 mL), 100% methanol (20 mL) to produce fractions A (48 mg), B (90 mg) and C (204 mg). Fraction A was injected into a semi preparative HPLC with a gradient from 2% to 10% solvent B in 2 min, 10% to 80% solvent B in 18 min, 80% to 100% solvent B in 1 min (solvent A: water; solvent B: acetonitrile) to collect the compounds 5-methylresorcinol (**1**, 2.7 mg), 5-propylresorcinol (**2**, 5.2 mg), methyl 2,4-dihydroxyl-6-methylbenzoate (**3**, 3.2 mg) and lecanoric acid (**6**, 2.2 mg) at t_R 10.9, 14.2, 15.7 and 16.4 min, respectively. Fraction B was separated by semi-preparative HPLC using a gradient from 2% to 30% solvent B in 2 min, 30% to 50% solvent B in 20 min (solvent A: water; solvent B: acetonitrile) to isolate methyl 2,4-dihydroxyl-6-methylbenzoate (**3**, 4.3 mg), lecanoric acid (**6**, 26.3 mg), 2,4-dihydroxyl-6-pentylbenzoate (**5**, 6.2 mg) and 3′-hydroxyl-5′-propylphenyl 2-hydroxyl-4-methoxy-6-methylbenzoate (**8**, 4.9 mg) at t_R 11.4, 15.4, 16.3, 23.5 min, respectively. Fraction C was injected into a semi-preparative HPLC with a gradient from 2% to 50% solvent B in 2 min, 50% to 100% solvent B in 16 min (solvent A: water; solvent B: acetonitrile, flow rate 3 mL/min) to produce lecanoric acid (**6**, 18.3 mg), 3′-hydroxyl-5′-propylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**10**, 6.2 mg), 3′-hydroxyl-5′-pentylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**11**, 7.9 mg), 3′-methoxyl-5′-propylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**13**, 2.6 mg), 3′-hydroxy-5′-propylphenyl 2-hydroxyl-4-methoxyl-6-pentylbenzoate (**12**, 1.4 mg), at t_R 8.5, 13.5, 15.9, 17.6 and 18.4 min, respectively. A fraction collected at t_R 11–13 min was repurified through further semi-preparative HPLC using gradient development from 2% to 20% solvent B in 2 min, 20% to 100% solvent B in 20 min (solvent A: water; solvent B: acetonitrile) to obtain 3′-hydroxyl-5′-pentylphenyl 2-hydroxyl-4-methoxyl-6-methylbenzoate (**7**, 0.7 mg) and 3′-hydroxyl-5′-methylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (**9**, 1.4 mg) at t_R 17.5, 17.8 min, respectively.

3.3.1. 3′-Hydroxyl-5′-pentylphenyl 2,4-dihydroxyl-6-methylbenzoate (7)

UV active, brown amorphous solid (0.7 mg, 0.01 mg g^{-1} dry wt); λ_{max} 216 (23,386) 269 (11,613); IR [cm^{-1}]: 3336 (m), 2927 (m) 1587 (s), 1251 (s), 1133 (s); For ^{1}H-NMR ($(CD_3)_2CO$, 600 MHz) and ^{13}C-NMR ($(CD_3)_2CO$, 150 MHz) spectroscopic data see Table S24; ESIMS$^-$, *m/z* 329 $[M-H]^-$. HRESIMS: calculated for $C_{19}H_{21}O_5$ $[M-H]^-$: 329.1394, found 329.1391.

3.3.2. 3′-Hydroxyl-5′-propylphenyl 2,4-dihydroxyl-6-methylbenzoate (8)

UV active, pale brown amorphous solid (4.9 mg, 0.11 mg g^{-1} dry wt); λ_{max} 212 (18,177) 269 (8,819) 301 (3,708); IR [cm^{-1}]: 3345 (m), 2957 (m), 1656 (s), 1615 (s) 1246 (s), 1156 (s); For ^{1}H-NMR ($(CD_3)_2CO$, 600 MHz) and ^{13}C-NMR ($(CD_3)_2CO$, 150 MHz) spectroscopic data see Table S24; ESIMS$^-$, *m/z* 301 $[M-H]^-$. HRESIMS: calculated for $C_{17}H_{17}O_5$ $[M-H]^-$: 301.1081, found 301.1079.

3.3.3. 3′-Hydroxyl-5′-methylphenyl 2-hydroxyl-4-methoxyl-6-propylbenzoate (9)

A UV active, brown amorphous solid (1.4 mg, 0.03 mg g^{-1} dry wt); λ_{max} 217 (18,502) 245 (6,242) 268 (8,607) 304 (3,557); IR [cm^{-1}]:3307 (m), 2931 (m), 1615 (s), 1248 (s), 1156 (s), 1019 (s); For ^{1}H-NMR ($(CD_3)_2CO$, 600 MHz) and ^{13}C-NMR ($(CD_3)_2CO$, 150 MHz) spectroscopic data see Table S24; ESIMS$^-$, *m/z* 315 $[M-H]^-$. HRESIMS: calculated for $C_{18}H_{19}O_5$ $[M-H]^-$: 315.1238, found 315.1237.

3.4. Antimicrobial assay

Compounds **1**–**13** (100 μM) were tested against the gram-negative bacterium *Aliivibrio fischeri* with chloramphenicol (100 μM) used as a positive control. Due to the special cell envelope structure of gram-negative bacteria the comparative high test concentration of 100 μM was applied. The bioactivity testing was performed using the *A. fischerii* strain DSM507 (batch no. 1209). The bacterial stock was cultured in BOSS medium (25 mL) at 100 rpm and 23 °C for 18 h. Dilution using fresh BOSS medium was necessary to gain a suitable cell number based on luminescence value (30,000–50,000 RLU (relative luminescence units)). The assay was performed on black flat bottom 96 well plates (CellGrade^{TM}premium, STERILE R) and consisted of bacterial solution (100 μL) and test solution (100 μL) with a final DMSO concentration of 1% in BOSS medium. The well plates were incubated under light absence at 23 °C and 100% humidity without lid and without shaking for 24 h. The luminescence was recorded using a microplate reader TecanSpark. The whole wavelength range was detected for 1000 ms without preliminary shaking to avoid secondary oxygen effects. Percentage inhibition was calculated as a relative value in comparison with the negative control (bacterial growth, 1% DMSO, without test compound). The results were obtained as an average of six replicate wells distributed on two well plates. Negative values represent an elevation of luminescene indicating increased bacterial growth.

3.5. Anthelmintic assay

The Bristol N2 wildtype strain of *Caenorhabditis elegans* was obtained from the Caenorabditis Genetic Center, University of Minnesota, Minneapolis, USA.

General procedures for preparing worms and the food source *E. coli* OP50 were based on a standard protocol (Stiernagle 2006). The assay is performed in 384 microtiter plates by counting living and dead worms in each well after 30 min incubation as previously described by Thomsen et al. (2012). Compounds **1**–**13** were tested in the concentration of 100 μg/mL. The solvent DMSO (2%) and the standard anthelmintic drug ivermectin (10 μg/mL) were used as negative and positive control, respectively. All assays were performed in triplicate. The results are expressed as percentage of dead worms ± SD.

4. Conclusion

The foliose lichen *Parmelia cetrata* from Indonesia produced typical depsides as secondary metabolites. A preparative HPLC isolation protocol enabled to obtain thirteen depside derivatives (**1**–**13**) including three new compounds (**7**–**9**). Pharmacological

studies indicate compound **6** to possess the highest antimicrobial activity against the gram-negative bacterium *Aliivibrio fischeri* with an inhibition value of 100% at a concentration of 100 μM. At the same concentration this compound also induced >80% mortality of *Caenorhabditis elegans* in an anthelmintic assay. The observed activities might be due to its free carboxylate group, not found in the other phenols. The new compounds **7** and **8** which have a free phenolic group at C4 caused 89.0% and 95.5% inhibition of bacterial growth at 100 μM, respectively. In summary, the lichen *P. cetrata* can be considered as valuable source for anti-infective natural products.

Acknowledgements

ASN thanks Mr. Aang Yudianto for assisting the field trip to collect the lichen in the high altitude region of Klocing-Bondowoso Indonesia. ASN also thanks the University of Jember and the Leibniz Institute of Plant Biochemistry, Halle (Germany), for research support. Furthermore, the authors acknowledge technical assistance by Gudrun Hahn (NMR), Anke Dettmer (bioassays) and Anja Ehrlich (HPLC).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The work was financially supported by BMBF (16GW0123) and DAAD (57243965) within the program 'Biodiversity and Health'.

ORCID

Ari Satia Nugraha http://orcid.org/0000-0002-9117-4713
Andrea Porzel http://orcid.org/0000-0002-8148-6895

References

Ango YP, Kapche G, Kuete V, Mapitse R, Yeboah SO, Ngadjui BT. 2016. Three new derivatives and others constituents from the roots and twigs of *Trilepisium madagascariense* DC. Helv Chim Acta. 99(8):642–649.

Brodo IM, Sharnoff SD, Sharnoff S. 2001. Lichens of North America. New Haven: Yale University Press; p. 1–795.

Burkholder PR, Evans AW, McVeigh I, Thornton HK. 1944. Antibiotic activity of lichens. Proc Natl Acad Sci USA. 30(9):250–255.

Celenza G, Segatore B, Setacci D, Perilli M, Brisdelli F, Bellio P, Piovano M, Garbarino JA, Amicosante G, Nicoletti M. 2013. Antibacterial activity of selected metabolites from *Chilean lichen* species against methicillin-mesistant *Staphylococci*. Nat Prod Res. 27(17):1528–1531.

Chan TH, Brownbridge P. 1980. Chemistry of enol silyl ethers. 5. A novel cycloaromatization reaction. Regiocontrolled synthesis of substituted methyl salicylates. J Am Chem Soc. 102(10): 3534–3538.

Cho JY, Moon JH, Seong KY, Park KH. 1998. Antimicrobial activity of 4-hydroxybenzoic acid and trans 4-hydroxycinnamic acid isolated and identified from rice hull. Biosci Biotechnol Biochem. 62(11):2273–2276.

Elix JA, Wardlaw JH. 1997. New depsides from the lichen *Neofuscelia depsidella*. Aust J Chem. 50(12):1145–1150.

Gianini AS, Marques MR, Carvalho NCP, Honda NK. 2008. Activities of 2,4-dihydroxy-6-n-pentyl-benzoic acid derivatives. Z Naturforsch C. 63(1–2):29–34.

Hew CS, Gam LH. 2010. The identification of high abundant proteins in the leaves of *Gynura Procumbens*. Biotechnol Biotechnol Equip. 24(4):2132–2136.

Ivanova V, Bačkor M, Dahse HM, Graefe U. 2010. Molecular structural studies of lichen substances with antimicrobial, antiproliferative, and cytotoxic effects from *Parmelia Subrudecta*. Prep Biochem Biotechnol. 40(4):377–388.

Lin H, Annamalai T, Bansod P, Tse-Dinh YC, Sun D. 2013. Synthesis and antibacterial evaluation of anziaic acid and its analogues as topoisomerase I inhibitors. MedChemComm. 4(12):1–14.

Merrill GK. 1913. Lichen from Java. Torreya. 13(6):133–137.

Molnár K, Farkas E. 2010. Current results on biological activities of lichen secondary metabolites: a review. Z Naturforsch C. 65(3–4):157–173.

Müller K. 2001. Pharmaceutically relevant metabolites from Lichens. Appl Microbiol Biotechnol. 56(1–2):9–16.

Negi HR. 2003. Lichens: A valuable bioresource for environmental monitoring and sustainable development. Reson. 8(1):51–58.

Nugraha AS, Keller PA. 2011. Revealing indigenous Indonesian traditional medicine: anti-infective agents. Nat Prod Commun. 6(12):1953–1966.

Nugraha AS, Pratoko DK, Damayanti YD, Lestari ND, Laksono TA, Addy HS, Untari LF, Kusumawardani B, Wangchuk P. 2019. Antibacterial and anticancer activities of nine lichens of Indonesian Java Island. J Biol Active Prod. 9(1):39–46.

Nugraha AS, Wangchuk T, Willis AC, Haritakun R, Sujadmiko H, Keller PA. 2019. Phytochemical and pharmacological studies on four Indonesian epiphytic medicinal plants: *Drynaria rigidula*, *Hydnophytum formicarum*, *Usnea misaminensis*, and *Calymperes schmidtii*. Nat Prod Commun. 14(6):1–6.

Schmeda-Hirschmann G, Tapia A, Lima B, Pertino M, Sortino M, Zacchino S, De Arias AR, Feresin GE. 2008. A new antifungal and antiprotozoal depside from the Andean lichen *Protousnea poeppigii*. Phytother Res. 22(3):349–355.

Selbmann L, Zucconi L, Ruisi S, Grube M, Cardinale M, Onofri S. 2010. Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance. Polar Biol. 33(1):71–83.

Shrestha G, Raphael J, Leavitt SD, St Clair LL. 2014. In vitro evaluation of the antibacterial activity of extracts from 34 species of North American lichens. Pharm Biol. 52(10):1262–1266.

Shrestha G, St Clair LL. 2013. Lichens: A promising source of antibiotic and anticancer drugs. Phytochem Rev. 12(1):229–244.

Stiernagle T. 2006. Maintenance of C. elegans. In: Hobert O, Editor. WormBook: The online review of *C. elegans* biology. Minneapolis: University of Minnesota;p. 1–11.

Thomsen H, Reider K, Franke K, Wessjohann LA, Keiser J, Dagne E, Arnold N. 2012. Characterization of constituents and anthelmintic properties of *Hagenia abyssinica*. Sci Pharm. 80(2):433–446.

Vagel A, Roo E. 2004. Alkylresorcinols – rare chemicals available in bulk. Innov Pharm Tech. 94–95.