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Chemical Profile of Compounds from Lichens of Bukit Larut, Peninsular Malaysia

(Profil Kimia Sebatian daripada Liken Bukit Larut, Semenanjung Malaysia)

LAILY B. DIN*, ZURIATI ZAKARIA, MOHD WAHID SAMSUDIN & JOHN A. ELIX

ABSTRACT

The lichen collection from Bukit Larut, Taiping, Malaysia in 1999 included Bulbothrix isidiza, Chrysothrix xanthina, Cladonia adspersa, C. verticillata, Coccocarpia palmicola, Heterodermia flabellata, H. japonica, H. obscurata, Hypotrachyna imbricatula, Leptogium azureum, Parmelinella wallichiana, Parmotrema tinctorum, P. clavuliferum, P. reticulatum, Pertusaria sp., Physma byrsaeum, Usnea baileyi *and* Usnea rubrotincta. *Secondary metabolites could not be detected in three lichens,* Coccocarpia palmicola, Leptogium azureum *and* Physma byrsaeum *by HPLC and TLC analysis. The other 15 lichen species showed the presence of ten classes of compounds, depsides (10 compounds), depsidones (16), quinones (5), xanthones (2), naphthopyrones (1), pulvinic acid derivatives (1), diphenylethers (1), dibenzofurans (1), aliphatic acids (4) and terpenoids (3).*

Keywords: Bukit Larut; lichen

ABSTRAK

Koleksi liken dari Bukit Larut, Taiping Malaysia pada 1999 merangkumi Bulbothrix isidiza, Chrysothrix xanthina, Cladonia adspersa, C. verticillata, Coccocarpia palmicola, Heterodermia flabellata, H. japonica, H. obscurata, Hypotrachyna imbricatula, Leptogium azureum, Parmelinella wallichiana, Parmotrema tinctorum, P. clavuliferum, P. reticulatum, Pertusaria sp., Physma byrsaeum, Usnea baileyi *dan* Usnea rubrotincta. *Daripada analisis HPLC dan TLC, metabolit sekunder tidak dapat dikesan dalam tiga liken iaitu* Coccocarpia palmicola, Leptogium azureum *dan* Physma byrsaeum. *Lima belas spesies liken yang lain menunjukkan kehadiran 10 kelas sebatian iaitu depsida (10 sebatian), depsidon (16), kuinon, (5), xanthon (2), naftopiron (1), terbitan asid pulvinik (1), difenileter (1), dibenzofuran (1), asid alifatik (4) dan terpenoid (3).*

Kata kunci: Bukit Larut; liken

INTRODUCTION

The distribution of lichen species is found to be more diverse in the mountainous areas of Malaysia (Din et al. 1998). Bukit Larut in Perak is regarded as a good location for lichen growth due to its location as well as the climatic conditions. Situated 9 km east of Taiping, 4°50'N, 100° 48'E and 1035 m above sea level, Bukit Larut is a hill resort noted for its gardens and is surrounded by tropical mountain rainforest. During a field survey conducted on 26 June 1999 by the UKM lichen group, a total of 23 lichen species were collected.

Five of the lichens, *Heterodermia appendiculata* (Korok) Swinscow & Krog (BLT 1), *Hypotrachyna ikomae* (Asahina) Hale (BLT 28 & BLT 36), *H. toiana* Elix (BLT 13, BLT 39 & BLT 46), *Parmotrema rampoddense* (Nyl.) Hale (BLT43), and *Stereocaulon coniophyllum* Lamb (BLT 17) were new records for Malaysia and their chemistry has been reported in *Australasian Lichenology* (Din et al. 2004).

MATERIALS AND METHODS

The voucher specimens were given BLT numbers (Bukit Larut) and deposited in the Herbarium Universiti Kebangsaan Malaysia (UKMB).

The 18 specimen analysed included *Bulbothrix isidiza* (Nyl) Hale (BLT 5), *Chrysothrix xanthina* (Vain.) Kalb (BLT 56), *Cladonia adspersa* Mont & Bosch (BLT 3, BLT 38 & BLT 42), *C. verticillata* Hoffm. (BLT 57), *Coccocarpia palmicola* Arvids & D.J Galloway (BLT 27), *Heterodermia flabellata* (Fée) Awasthi (BLT 45), *H. japonica* (Sato) Swinscow & Krog (BLT 10), *H. obscurata* (Nyl.) Trev. (BLT 34 & BLT 53), *Hypotrachyna imbricatula* (Zahlbr) Hale (BLT 32), *Leptogium azureum* (Ach.) Mont (BLT 49), *Parmelinella wallichiana* (Taylor) Elix and Hale (BLT 20), *Parmotrema clavuliferum* (Räsänen) Streim. (BLT 33, BLT 37, BLT 40 & BLT 44), *P. reticulatum* (BLT 52), *P. tinctorum* (Nyl.) Hale (BLT 29), *Pertusaria* sp. (BLT 51), *Physma byrsaeum* (Ach.) Müll. Arg (BLT 55), *Usnea baileyi* (Stirt.) Zahlbr. (BLT 22) and *Usnea rubrotincta* Stirt. (BLT 14, BLT 16 & BLT 18).

The lichen fragments were freed as far as possible from any trace of organic substratum, and the sample (0.5 g for each lichen) was extracted with warm acetone for thin layer chromatography (TLC) using Merck Kieselgel 60 F_{258} plates (20 × 20 cm^2) as the stationary phase, and the solvent system C (toluene:acetic acid = 200:30). The spots (compounds) on the developed TLC plates were detected under ultraviolet light (λ_{254} nm) and by spraying with 10% H_2SO_4 followed by heating on a hot plate. The compounds were characterized using the methods standardized for lichen products (Culberson 1972; Culberson & Johnson 1982; Elix & Ernst-Russell 1993).

For high performance liquid chromatography (HPLC), a Hewlett Packard HP 1050 Series System, a Phenomenex Hypersil 5µ C18 column (250 by 4.6 mm) with a flow rate of 1mL/min were used. Two solvent systems were used: 1% aqueous orthophosphoric acid and methanol in the ratio 7:3 (A) and methanol (B). The run started with 100% A and was raised to 58% B within 15 min., then to 100% B within a further 16 min, followed by isocratic elution in 100% B for a further 10 min. Compounds were identified with retention index values (R_t) calculated from benzoic acid and solorinic acid controls (Feige et al. 1993). The HPLC was coupled to a photodiode detector for ultraviolet spectroscopic comparisons. By this means the ultraviolet spectra observed for the various component eluting in the HPLC chromatogram were recorded and computer-matched against a library of ultraviolet spectra recorded for authentic metabolites under identical conditions. For each substance the correlation of the ultraviolet spectra of the synthetic and natural material was greater than 99.9%.

Authentic samples for the analysis were provided by the lichen laboratory, Australian National University, Canberra, Australia.

Results and Discussion

The lichen flora of Bukit Larut was noticeably more diverse than that observed in the Cameron Highlands and Fraser's Hill, probably due to the fact that Bukit Larut is more humid, the slopes are more exposed to sunlight and the area less disturbed by development. Such lichen diversity is also observed at Mount Kinabalu and at Bario in the Kelabit Highlands of Sarawak (Din et al. 1998; Sipman 1993). *Usnea* species were found to be abundant hanging down from trees as well as electric cables and telephone lines. Other lichens were collected from hill slopes, tree trunks and rocks.

The lichens were extracted with acetone and the TLC analyses particularly with the triterpenes were carried out. Results of TLC analysis are listed in Table 1.

Triterpenes were observed for only the *Heterodermia* species. The triterpene content for *Heterodermia flabellata*, *H. japonica* and *H. obscurata* were similar to that reported for *H. appendiculata* (Din et al. 2004). Comparison of R_f values with authentic samples as well as (R_f ×100) values from the literature (Culberson 1972) showed that the triterpenes present were zeorin, 6α-acetoxyhopane-16β,22-diol, and 16β-acetoxyhopane-6α, 22-diol, solvent. Previous study on *H. flabellata* from Gunung Jerai showed atranorin was the major component whilst zeorin was the minor component (Din et al. 2002). *Parmotrema tinctorum* contained only atranorin and lecanoric acid and *Pertusaria sp* contained stictic and constictic acid.

The retention times of the separated components by HPLC analysis were compared to the authentic samples from the lichen laboratory at Australian National University.

Both TLC and HPLC analyses showed that secondary metabolites were absent in three species, *Coccocarpia palmicola, Leptogium azureum* and *Physma byrsaeum*.

TABLE 1. TLC analysis of lichens from Bukit Larut, Taiping

Species	Specimen No	Retention Factor (R_f ×100)	Secondary Metabolite
Heterodermia appendiculata	BLT 1*	34	6α-acetoxyhopane-16β,22-diol
		36	16β-acetoxyhopane-6α,22-diol
		43	zeorin
Heterodermia flabellata	BLT 45	34	6α-acetoxyhopane-16β,22-diol
		36	16β-acetoxyhopane-6α,22-diol
		43	zeorin
Heterodermia japonica	BLT 10	34	6α-acetoxyhopane-16β,22-diol
		36	16β-acetoxyhopane-6α,22-diol
		43	zeorin
Heterodermia obscurata	BLT 34	34	6α-acetoxyhopane-16β,22-diol
		36	16β-acetoxyhopane-6α,22-diol
		43	zeorin
Parmotrema tinctorum	BLT 29	22	lecanoric acid (major)
		78	atranorin
Pertusaria sp.	BLT 51	18	stictic acid (major)
		2	constictic acid

TLC was developed in Solvent C toluene : acetic acid (200:30) (Culberson 1972)

The other 15 species exhibited the presence of ten classes of compounds, depsides (i) (10 compounds), depsidones (ii) (16), quinones (iii) (5), xanthones (iv) (4), naphthopyrones (v) (2), pulvinic acid derivatives (vi) (1), diphenyl ethers (vii) (1), dibenzofurans (viii) (1), aliphatic acids (ix) (5) and terpenoids (x) (3). Results of the HPLC analysis are shown in Table 2. The classes and structures of the chemical components are shown in Figure 1.

TABLE 2. HPLC analysis of lichens from Bukit Larut, Taiping

Species	Specimen No	Retention Times (min)	Secondary Metabolite
Bulbothrix isidiza	BLT 5	10.66	consalazinic acid
		15.63	salazinic acid,
		29.42	atranorin
Chrysothrix xanthina	BLT 56	26.19	pinastric acid (major)
		29.41	atranorin
Cladonia adspersa	BLT 3	18.56	confumarprotocetraric acid
		20.15	protocetraric acid
		22.81	fumarprotocetraric acid
		26.65	sekikaic acid,
		28.56	homosekikaic acid(major)
		29.40	atranorin
		30.12	hyperhomosekikaic acid
Cladonia verticillata	BLT 57	15.66	salazinic acid
		18.25	confumarprotocetraric acid
		20.05	protocetraric acid,
		22.70	fumarprotocetraric acid(major)
		25.30	gyrophoric acid
		28.43	usnic acid
		29.27	atranorin
Cococarpia palmicola	BLT 27		no lichenic substances
Heterodermia appendiculata	BLT 1*	14.50	connorstictic acid
		15.78	salazinic acid
		19.41	norstictic acid(major)
		26.12	chloroatranoric acid
		27.56	constipatic acid
		29.41	atranorin (major)
		30.50	chloroatranorin
Heterodermia flabellata	BLT 45	27.50	emodin
		28.70	7-chloroemodin
		29.40	atranorin,
		30.95	flavo-obscurin A,
		31.47	flavo-obscurin B1
		31.63	flavo-obscurin B2
Heterodermia japonica	BLT 10	14.51	connorstictic acid
		15.79	salazinic acid
		19.43	norstictic acid (major)
		27.58	constipatic acid
		29.42	atranorin
Heterodermia obscurata	BLT 34	27.50	emodin
		28.70	7-chloroemodin
		29.40	atranorin (major)
		30.95	flavo-obscurin A,
		31.47	flavo-obscurin B1
		31.63	flavo-obscurin B2
Hypotrachyna ikomae	BLT 32 *	27.91	nephrosterinic acid
		28.48	isonephrosterinic acid
		29.39	atranorin (major)
		30.08	protolichesterinic acid
		30.47	chloroatranorin +lichesterinic acid

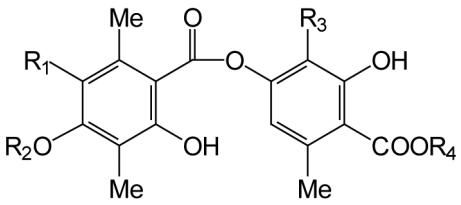
(*continue*)

Continued (TABLE 2)

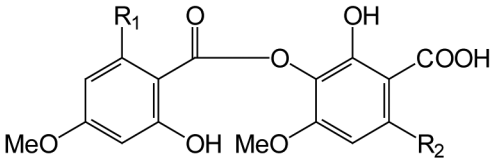
Species	Specimen No	Retention Times (min)	Secondary Metabolite
Hypotrachyna imbricatula	BLT 36	23.72	norobtusatic acid (major)
		25.26	4-*O*-demethylbarbatic acid (major)
		27.88	obtusatic acid
		29.18	barbatic acid (major)
		29.40	atranorin
		30.48	chloroatranorin,
Hypotrachyna toiana	BLT 39 *	25.26	4-*O*-demethylbarbatic acid (major)
		26.27	vioxanthin
		26.44	pigmentosin A
		27.89	obtusatic acid
		29.17	barbatic acid (major)
		29.39	atranorin
		30.48	chloroatranorin
		31.39	skyrin
Leptogium azureum	BLT 49		no lichen substances
Parmelinella wallichiana	BLT 20	10.71	consalazinic acid
		15.70	salazinic acid (major)
		29.34	atranorin
Parmotrema clavuliferum	BLT33	10.72	consalazinic acid
		15.70	salazinic acid (major)
		29.36	atranorin
		30.43	chloroatranorin
Parmotrema rampoddense	BLT 43*	24.99	β-alectoronic acid
		25.64	alectoronic acid
		27.21	α-collatolic acid
		29.40	atranorin
		30.49	chloroatranorin
		31.75	skyrin
Parmotrema reticulatum	BLT 52	10.77	consalazinic acid
		15.75	salazinic acid (major)
		25.65	gyrophoric acid
		29.40	atranorin
		30.48	chloroatranorin
Stereocaulon coniophyllum	BLT 17*	25.63	oxolobaric acid
		26.35	sublobaric acid
		27.65	lobaric acid
		29.54	atranorin
Usnea baileyi	BLT 19	15.79	salazinic acid (major)
		19.44	norstictic acid
		20.19	protocetraric acid
		23.91	eumitrin B2
		24.30	eumitrin A3(major)
		25.30	eumitrin B1
		25.40	eumitrin A1
		28.55	usnic acid (major)
		29.42	atranorin
Usnea rubrotincta	BLT 18	15.86	salazinic acid(major)
		19.49	norstictic acid (major)
		20.52	protocetraric acid
		25.77	virensic acid
		28.58	usnic acid
		29.46	atranorin

* Published in Din et al. 2004

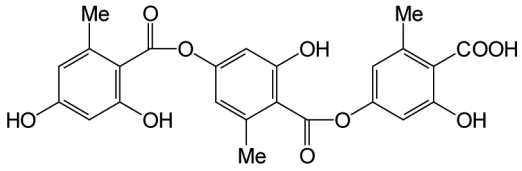
i. Depsides



Atranorin	R_1=H, R_2=H, R_3=Me, R_4=Me
Chloroatranorin	R_1=Cl, R_2=H, R_3=Me, R_4=Me
Barbatic acid	R_1=H, R_2=Me, R_3=Me, R_4=H
4-*O*-Demethylbarbatic acid	R_1=H, R_2=H, R_3=Me, R_4=H
Obtusatic acid	R_1=H, R_2=Me, R_3=H, R_4=H
Norobtusatic acid	R_1=H, R_2=H, R_3=H, R_4=H

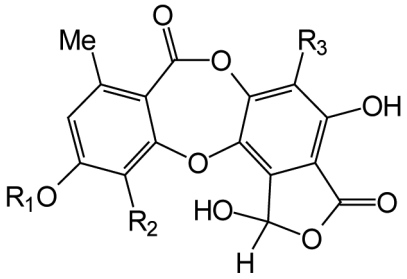


Sekikaic acid	$R_1 = C_3H_7$-, $R_2 = C_3H_7$-
Homosekikaic acid	$R_1 = C_3H_7$-, $R_2 = C_5H_{11}$-
Hyperhomosekikaic acid	$R_1 = C_5H_{11}$-, $R_2 = C_5H_{11}$,

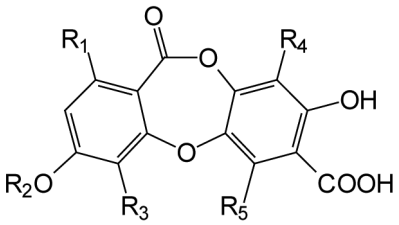


Gyrophoric acid

ii. Depsidones



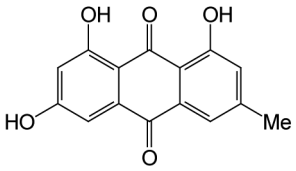
Salazinic acid	R_1= H, R_2=CHO, R_3=CH_2OH
Consalazinic acid	R_1= H, R_2=CH_2OH, R_3=CH_2OH
Stictic acid	R_1= Me, R_2=CHO, R_3=Me
Constictic acid	R_1= Me, R_2=CHO, R_3= CH_2OH
Norstictic acid	R_1= H, R_2=CHO, R_3= Me
Connorstictic acid	R_1= H, R_2= CH_2OH, R_3= Me



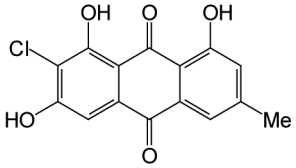
Virensic acid	R_1=CH_3, R_2=H, R_3=CHO, R_4=CH_3, R_5= CH_3
Protocetraric acid	R_1=CH_3, R_2=H, R_3=CHO, R_4=CH_2OH, R_5= CH_3
Fumarprotocetraric acid	R_1= CH_3-, R_2=H, R_3=CHO, R_4=HOOCCH=CHCOOCH$_2$-, R_5= CH_3
Confumarprotocetraric acid	R_1= CH_3-, R_2=H, R_3=CH_2OH, R_4=HOOCCHCHCOOCH$_2$-, R_5= CH_3
Lobaric acid	R_1= C_4H_9CO-, R_2=CH_3, R_3=H, R_4=H, R_5= C_5H_{11}
Sublobaric acid	R_1= C_2H_5CO-, R_2=CH_3, R_3=H, R_4=H, R_5= C_5H_{11}
Oxolobaric acid	R_1= $CH_3COCH_2CH_2CO$-, R_2=CH_3, R_3=H, R_4=H, R_5= C_5H_{11}
Alectoronic acid	R1 = $C_5H_{11}COCH_2$-, R_2=H, R_3=H, R_4=H, R_5= $C_5H_{11}COCH_2$-
α-Collatolic acid	R_1= $C_5H_{11}COCH_2$-, R_2= CH_3, R_3=H, R_4=H, R_5= $C_5H_{11}COCH_2$-,

FIGURE 1. (*continue*)

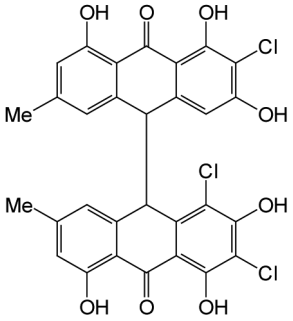
iii. Quinones



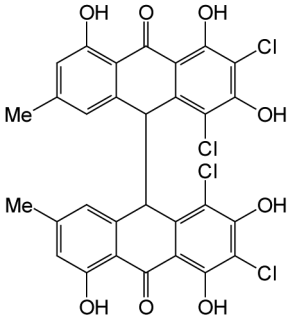
Emodin



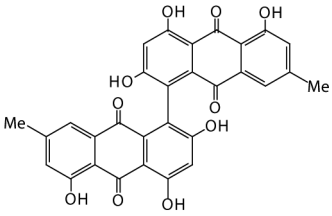
7-Chloroemodin



Flavo-obscurin A

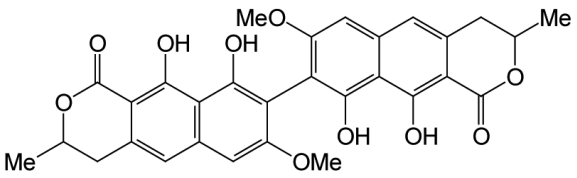


Flavo-obscurin B

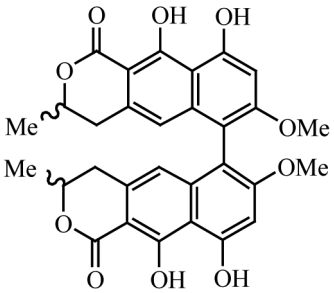


Skyrin

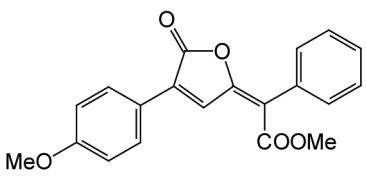
iv. Xanthones (ergochromes)



Vioxanthin

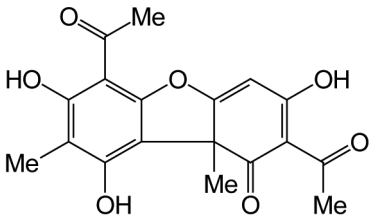


vi. Pulvinic Acid Derivatives



Pinastric acid

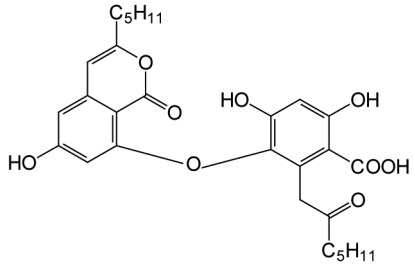
vii. Dibenzofurans



Usnic acid

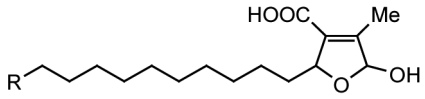
FIGURE 1. (*continue*)

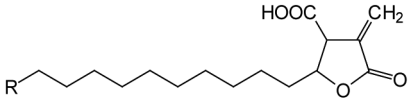
viii. Dephenyl ethers



Usnic acid

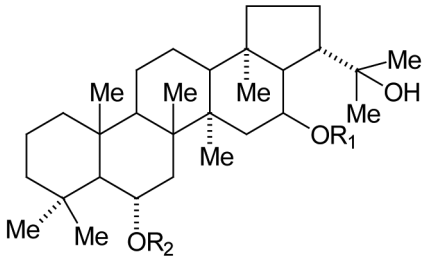
ix. Aliphatic Acids

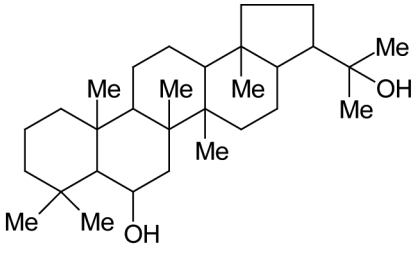




Constipatic acid	R = $CH_3CHOHCH_2CH_2CH_2$ -	Protolichesterinic acid	R=C_3H_7-
Lichesterinic acid	R= $CH_3CH_2CH_2$-	Nephrosterinic acid	R = CH_3-
Isonephrosterininic acid	R=CH_3-		

x. Terpenoids





16β-acetoxyhopane-6α,22-diol	R_1= H, R_2=COMe	Zeorin
6α-acetoxyhopane-16β,22-diol	R_1=COMe, R_2=H	

FIGURE 1. Structures of Compounds from Lichens of Bukit Larut

Bulbothrix isidiza showed three compounds comprising consalazinic acid, salazinic acid and atranorin.

Chrysothriz xanthina, previously known as *C. candelaris,* contained atranorin and pinastric acid which is the major compound. Pinastric acid was also reported present in *C. candelaris* obtained from the bark of the Semarak tree *Delonix regia* found in the low lying area of Petaling Jaya, Selangor (Din et al. 1991).

Seven compounds were detected for both *Cladonia adspersa* and *C. verticillata*. Common to both species are confumarprotocetraric acid, protocetraric acid, fumarprotocetraric acid and atranorin. However, the major compound for *C. adspersa* is homosekikaic acid and for *C. verticillata*, the major compound is fumaprotocetraric acid. The *Cladonia* species vary with its content as previous report of *Cladonia* from Cameron Highlands showed that the major compound for *C. vulcanica* is condidymic acid (Samsudin et al.1987) and *C. micalenta* is rhadocladonic acid (Latiff et al.1988).

From the HPLC results, the *Heterodermia* species from Bukit Larut can be grouped into two types. *H. appendiculata* and *H. japonica* belong to the first group as they have the same biomarkers which are connorstictic, salazinic, norstictic, constipatic acids and atranorin. *H. flabellata* and *H. obscurata* belong to the second group where anthraquinone derivatives are predominantly present (emodin, 7-chloroemodin, flavo-obscurins A, B1 and B2). All the *Heterodermia* species contain atranorin.

All the compounds in *Hypotrachyna imbricatula* except for norobtusatic acid are found in *H. toiana*. The major compounds in *H. imbricatula* are norobstusatic, 4-*O*-demethylbarbattic and barbatic acids. The same species collected from Australia contain the same compounds, but only barbatic acid was the major component whilst others are detected as minor or trace compounds (Elix 1994). Vioxanthin and pigmentosin A, yellow-green naphthopyrone derivatives present in *H. tioana* were not present in *H. imbricatula*. Although atranorin and

chloroatranorin are common to all *Hyptrachyna* in Bukit Larut, the chemical profile of *H. ikomae* is different as aliphatic acids nephrosterinic, isonephrosterinic, protolichesterinic acid and lichesterinic acids are present only in this species.

Parmelinella wallichiana contain consalazinic acid, salazinic acid (major) and atranorin.

Parmotrema clavuliferum and *P. reticulatum* contain similar compounds with salazinic acid as the major component. *P. rampoddense* contains β-alectoronic, alectoronic and α-collatolic acid which are not detected in the other *Parmotema* species .

Stereocaulon coniophyllum have already been described (Din et al. 2004). Four ergochromes (eumitrins A1. A3, B1 and B2) dimeric xanthone derivatives noted for their potent toxicity were found in *Usnea baileyi*. Aside from the ergochromes, other compounds in *U. baileyi* are also found in *U. rubrotincta*.

Conclusion

The lichen flora of Bukit Larut is more diverse when compared to other mountainous areas in Peninsular Malaysia, due to heavy rainfall and higher light exposure. For the chemical profiles of the lichens fron Bukit Larut, particularly noteworthy were vioxanthin and pigmentosin A, yellow-green naphthopyrone derivatives detected in the lichen *Hypotrachyna toiana* and ergochromes (eumitrins A1, A3, B1 and B2), detected in *Usnea baileyi*. *Heterodermia flabellata* and *H. obsurata* proved to be rich sources of anthraquinone derivatives (emodin, 7-chloroemodin, flavor-obscurins A, B1 and B2) while all three Heterodermia species produced triterpenes (zeorin, 6α-acetoxyhopane-16β, 22-diol, 16β-acetoxyhopene-6α, 22-diol). β-Alectoronic acid, the only diphenyl ether detected, was present in *Parmotrema rampoddense*. Aliphatic acids were detected in two species, *Heterodermia japonica* (constipatic acid) and *Hypotrachyna ikomae* (lichesterinic acid, protolichesterinic acid, nephrosterinic acid and isonephrosterinic acid). The other lichen substances including all the depsides, depsidones, skyrin, usnic acid and pinastric acid are common and widely distributed in many lichen species.

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Laily B. Din*, Zuriati Zakaria & Mohd Wahid Samsudin
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 UKM Bangi
Selangor, Malaysia

John A. Elix
Department & Chemistry, Faculty of Science
Australian National University
ACT 0200
Canberra, Australia

*Corresponding author; email: lbdin@ukm.my

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