

Phytochemical and Pharmacological Studies on Four Indonesian Epiphytic Medicinal Plants: *Drynaria rigidula*, *Hydnophytum formicarum*, *Usnea misaminensis*, and *Calymperes schmidtii*

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Abstract

Phytochemical studies performed on 4 Indonesian epiphytic medicinal plants, *Drynaria rigidula* L., *Hydnophytum formicarum* Jack, *Usnea misaminensis* (Vain) Motyka, and *Calymperes schmidtii* Broth., revealed 11 known secondary metabolites (1–11), reported from these species for the first time. The methanol extracts and their fractions were screened against infective agents and cancer cells. The dichloromethane fractions of *H. formicarum*, *U. misaminensis*, and *C. schmidtii* showed activity against lung cancer cells with IC₅₀ values of 35, 11, and 20 µg/mL, respectively.

Keywords

Indonesian medicinal plant, epiphytes, isolation, *Drynaria rigidula*, *Hydnophytum formicarum*, *Usnea misaminensis*, *Calymperes schmidtii*

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The archipelago of Indonesia is rich in medicinal plants including the neglected subset of epiphytic medicinal plants.¹ Our previous studies revealed the chemical constituents, and their corresponding activities, from the rhizome of the Indonesian epiphytic fern *Drynaria rigidula* L.² Here we report on the continued phytochemical investigations into Indonesian epiphytic species including the leaves of *D. rigidula*, and further, the whole plant of *Hydnophytum formicarum* Jack, an epiphytic lichen *Usnea misaminensis* (Vain) motyka and an epiphytic bryophyte *Calymperes schmidtii* Broth (Figure 1). The indigenous people of Indonesia have claimed the medicinal benefits of these species including *D. rigidula* to treat eye infection, a decocted pith of *H. formicarum* drunken to alleviate swellings and headache, whereas *U. misaminensis* was traditionally used to treat fever, dysentery, inflammation, and hypertension. *Usnea* was claimed to have astringent, diuretic, antifatulent, and anti-inflammatory activities and the decocted plant was prescribed to treat diarrhea and dysentery, aphthous ulcers, and abdominal distention.³ *Calymperes schmidtii* is part of the nonvascular plant group, bryophytes, which consists of approximately 21 000 species, of which only 2% have been phytochemically studied.^{4,5} Compared to common medicinal plants, medicinal

uses of bryophytes are less common with reported traditional uses of mosses including the external use of a poultice of *Calymperes* sp. as insecticidal agent.⁶ These epiphytes reside in moist, dense clusters, which are likely to harvest microbes, and, therefore, these species may produce antimicrobial secondary metabolites. The limited reports of phytochemical

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Figure 1. Indonesian epiphytic medicinal plants. (a) *Drynaria rigidula* L, b) *Hynophyllum formicarum* Jack, c) *Usnea misaminensis* (Vain) Motyka and d) *Calymperes schmidtii* Broth.)

data analyzing these medicinal epiphytic plant, therefore, lead to this current investigation focusing on the chemical and bioactivity investigations of the extracts and fractions of these epiphytes. Specifically, we tested a range of biological activities; as examples, against *Plasmodium falciparum* given its prevalence in the region as well as the possibility of this microorganism being responsible for some of the symptoms treated in traditional medicine in the region, and against *Mycobacterium tuberculosis* as a surrogate for antimicrobial activity as well as being a microbe in which new treatments are still being investigated. We also tested against herpes simplex virus type 1, cancer cell lines, and cytotoxicity testing.

The phytochemical studies on these 4 Indonesian epiphytic medicinal plants resulted in the isolation of 11 secondary metabolites (Figure 2). The hexane fraction from the crude extract of the leaves of *D. rigidula* produced 3 major secondary metabolites, namely campesterol **1**, stigmasterol **2**, and

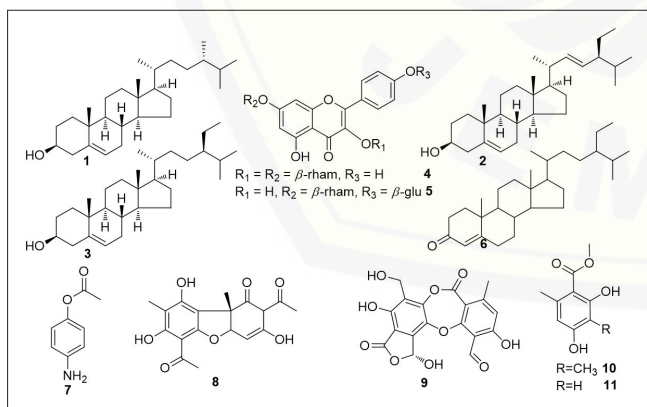


Figure 2. Secondary metabolites isolated from 4 Indonesian epiphytic medicinal plants, *Drynaria rigidula* (**1-3**, **4**, **5**), *Hynophyllum formicarum* (**3**, **6**, **7**), *Usnea misaminensis* (**8**, **9**), *Calymperes schmidtii* (**10**, **11**).

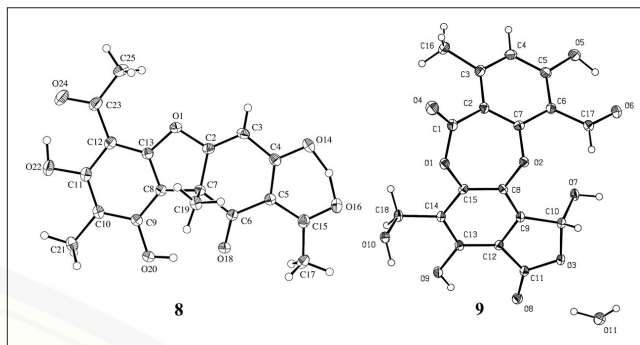


Figure 3. Oak ridge thermal-ellipsoid plot program drawing of usnic acid **8** and salazinic acid **9**.

β -sitosterol **3**. The molecular structures were deduced by comparison of their electron impact mass spectra against the NIST standard reference database. The corresponding dichloromethane fraction was subjected to silica column chromatography followed by semipreparative high-performance liquid chromatography (HPLC), and resulted in the isolation of compound **4**.² A separate semipreparative HPLC protocol was applied to the ethyl acetate fraction and the residue and gave 2 isolates, which were characterized as kaempferol-3,7-di-*O*- α -L-rhamnopyranoside **4**² and kaempferol-7-*O*- α -L-rhamnopyranosyl-4'-*O*-glucopyranoside **5** (spectral data supplied in Supplemental material).⁷ Compound **4** was also found in a 5-fold lower yield in our previous investigation of the rhizome *D. rigidula* (1.63 mg/g sample).²

Phytochemical investigations into the hexane fraction from the initial methanol extract of *H. formicarum* produced 2 sterols stigmasterol-4-en-3-one **6**⁸ and β -sitosterol **3**,⁹ and the aniline derivative 4-aminophenyl acetate **7** (spectral data supplied in Supplemental material). This is the first report of these 3 compounds being isolated from this species. Further analyses on the dichloromethane fraction were constrained due to limited yield. Analysis of the HPLC chromatogram of the brown shiny ethyl acetate fraction indicated no discernable peaks, even after back extraction with both acid and base.

The *n*-hexane fraction from the initial methanol extract of *U. misaminensis* produced pale yellow crystals, which were identified as usnic acid **8**,¹⁰ with a second crop obtained following back extraction with dichloromethane. Extraction with ethyl acetate followed by semipreparative HPLC resulted in the isolation and identification of salazinic acid **9**¹¹ as a white needle crystals. The structures of **8** and **9** were confirmed by X-ray crystallographic analysis (Figure 3) (spectral data supplied in Supplemental material).

Usnic acid and salazinic acid have been reported to possess antibacterial activities^{12,13} with the latter having inhibitory activity against the inflammation regulator, microsomal prostaglandin E_2 synthase-1.¹⁴ These outcomes support the

traditional claims of the Indonesian indigenous population with the use of *U. misaminensis* in infective-related dysentery and inflammation therapy.

Undertaking phytochemical studies on the moss *C. schmidtii* was challenging due to the limited availability of biomass. Therefore, its isolation protocol was simplified with the defatted crude extract directly subjected to semipreparative HPLC. A gradient eluent from 100% to 0% of solvent A (solvent A, 0.1% trifluoroacetic acid [TFA] in water; solvent B, 0.1% TFA in acetonitrile) within 80 minutes led to the isolation of 2 benzoic acid derivatives at retention times of 39 and 43 minutes. These major constituents (Figure 2) were assigned as methyl 2,4-dihydroxy-3,6-dimethylbenzoate **10**,¹⁵ and methyl 2,4-dihydroxy-6-methylbenzoate **11**,¹⁶ and this is the first report of these compounds being isolated from an epiphytic moss species (spectral data supplied in Supplemental material). It is the reported active component of the commercial herbal medicine Tadenan' which is used in therapy for benign prostate hyperplasia and in combination therapy for prostate cancer. Further studies revealed that the acid **10** inhibited prostate

cancer cell growth, acting as an antagonist of the human androgen receptor.¹⁷

The crude extracts from all 4 species, and their fractions, were screened against a small range of cancer cell lines, and for their antimicrobial activity (Table 1). The DCM fraction of *D. rigidula* was cytotoxic against breast cancer cell MCF-7 possibly due to the presence of kaempferitrin **4**, which was previously reported to have activity against the cell line with an half-maximal inhibitory concentration (IC₅₀) value of 0.062 mM.¹⁸ The hexane and DCM fractions of *H. formicarum* inhibited lung cancer NCI-H187 cell growth and are likely due to the component, β -sitosterol **3** being present, as it was previously reported to possess the same action with an IC₅₀ value of 26.45 μ g/mL.¹⁹ The DCM fraction of *U. misaminensis* showed broad cytotoxicity against KB cell, lung cancer cell NCI-H187 and breast cancer cell MCF-7 with IC₅₀ values of 28, 11, and 30 μ g/mL, respectively. The isolated constituent from this fraction, usnic acid **8**, was previously reported to induce apoptosis via a reactive oxygen scavenger-dependent mitochondrial pathway in MCF-7

Table 1. Bioactivity Testing Results of Crude Extracts and Their Fractions.

Species	Extract	Inhibition (IC ₅₀ , μ g/mL)						
		KB cells	Lung cancer NCI-H187	Breast cancer MCF-7	Vero cells	HSV-1	TB	Pf
<i>Drynaria rigidula</i>	Methanol	-	-	-	na	na	na	na
	<i>n</i> -Hexane	-	-	-	na	na	na	na
	Dichloromethane	-	-	18	na	na	na	na
	Ethyl acetate	-	-	-	na	na	na	na
	Residue	-	-	-	na	na	na	na
<i>Hynophyllum formicarum</i>	Methanol	-	-	27	-	-	-	-
	<i>n</i> -Hexane	-	18	-	49	-	-	-
	Dichloromethane	-	35	-	49	-	-	-
	Ethyl acetate	-	-	-	-	-	-	-
	Residue	-	-	-	-	-	-	-
<i>Usnea misaminensis</i>	Methanol	-	31	-	-	-	-	-
	<i>n</i> -Hexane	-	48	-	-	-	-	-
	Dichloromethane	28	11	30	20	-	50	-
	Ethyl acetate	-	-	-	-	-	-	-
	Residue	-	-	-	-	-	-	-
<i>Calymperes schmidtii</i>	Methanol	-	49	-	-	-	-	-
	Hexane	-	38	-	-	-	-	-
	Dichloromethane	-	20	-	-	48	-	-
	Ethyl acetate	-	49	-	-	-	-	-
	Residue	-	-	-	-	-	-	-

-, inactive; na, not available; HSV, Herpes simplex virus; TB, tuberculosis; Pf, *Plasmodium falciparum*. IC₅₀, half-maximal inhibitory concentration; IC50 positive control: Ellipticine = 1.47 μ g/mL against KB cell, 2.47 μ g/mL against lung cancer NCI-H187, 1.83 μ g/mL against vero cell; Tamoxifen = 6.13 μ g/mL against breast cancer MCF-7; Mafloquine = 0.029 μ g/mL against *Plasmodium falciparum*; Streptomycin = 0.156 μ g/mL against *Mycobacterium tuberculosis*; Acyclovir = 7.86 μ g/mL against HSV-1.

cells²⁰. Interestingly, the DCM fraction also showed that anti-mycobacterium activity with usnic acid **8** was previously reported with an MIC value of 62.5 µg/mL against *Mycobacterium tuberculosis* H37Rv.²¹ The crude extract and fractions from *C. schmidtii* inhibited lung cancer cell growth and HSV-1; however, compounds **10** and **11** have previously been reported for anticancer and anti-viral activities.

Conclusion

Studies on 4 Indonesian epiphytic medicinal plants, *D. rigidula*, *H. formicarum*, *U. misaminensis*, and *C. schmidtii*, successfully revealed 11 major constituents. Lung cancer cells NCI-H187 showed sensitivity against most of the crude methanol extracts and their subfractions with the exception of *D. rigidula*. The dichloromethane subfraction of *U. misaminensis* exerts cytotoxicity against KB-oral cancer cell, lung cancer cell NCI-H187, and breast cancer cell MCF-7.

Experimental

Plant Material

Epiphytic medicinal plant samples, *D. rigidula*, *U. misaminensis*, and *C. schmidtii*, were collected from Malabar forest surrounding Bondowoso, Indonesia during wet season on February 2010 and sample vouchers are deposited under accession codes DRL, UM, and BSp, respectively. *Hynophyllum formicarum* was purchased from the Traditional Market at Klaten, Jawa Tengah-Indonesia in which the sample originated from West Papua-Indonesia. The voucher sample of *H. formicarum* was deposited under accession number HF. The *Drynaria*, *Hynophyllum*, and *Usnea* were identified at Faculty of Pharmacy, University of Jember Indonesia. The *C. schmidtii* was identified by Mr Heri Sujadmiko, a bryologist at Faculty of Biology, Gadjah Mada University, Indonesia. Samples were cleaned, washed, and cut drying under sun shade. The dried samples were then separately pulverized using a grinding machine.

Extraction and Constituent Isolation of Leaves of *Drynaria rigidula*

To leaf powder (500 g) was added MeOH (5.0 L) and the mixture stirred for 48 hours. The suspension was filtered and the supernatant vacuum dried to produce crude leaves extract (48.1 g). Liquid-liquid fractionation from the leaves extract produced *n*-hexane (11.7307 g), DCM (2.3467 g), EtOAc (10.8527 g), and residual (22.9089 g) fractions.

The hexane fraction was prepared for gas chromatography mass spectrometry (GC-MS) analysis with samples (5 mg/mL) prepared by treating the solution with BSTFA (*N,O*-bis(trimethylsilyl)trifluoroacetamide) for 12 hours. The trimethylsilyl derivatives were then subjected to GC-MS and the resulting spectra compared with spectra in the NIST08 database. This revealed 3 major secondary metabolites,

namely campesterol **1**, stigmasterol **2**, and β -sitosterol **3**. The DCM fraction (1.8755 g) was subjected to a silica gel column chromatography plug (2 × 30 cm) and gradient elution with *n*-hexane, transitioning to EtOAc and EtOAc to ACN to produce 122 fractions. Analysis by thin-layer chromatography (TLC) allowed sample pooling to produce 13 fractions. The subfraction 10 (150 mg) was loaded into a short RP-column (1.5 × 3.0 cm) and washed with MeOH (50 mL) and EtOAc (100 mL). The relevant fraction(s) were then concentrated to 10 mL and subjected to semipreparative HPLC with a gradient elution from 100% to 50% of solvent A within 40 minutes (solvent A: 0.1% TFA in H₂O; solvent B: 0.1% TFA in ACN) to yield kaempferol-7-*O*- α -L-rhamnopyranosyl-4'-*O*-glucopyranoside **5** (20 mg). The EtOAc fraction (350 mg) was subjected to short silica gel column chromatography (2 cm × 5 cm) and elution with ACN:MeOH:H₂O (8:1:1) produced a 200 mL fraction, which was concentrated to 7 mL. The sample was then injected in 7 blocks in preparative HPLC with a gradient elution from 100% to 80% solvent A (0.1% TFA in H₂O) in 40 minutes with solvent B (0.1% TFA in acetonitrile) to produce kaempferitrin **4** (100 mg) and kaempferol-7-*O*- α -L-rhamnopyranosyl-4'-*O*-glucopyranoside **5** (28 mg).

Extraction and Constituent Isolation of *Hynophyllum formicarum*

A suspension of the whole plant powder (600 g) in MeOH (5.0 L) was stirred for 24 hours, filtered, and the supernatant vacuum dried to produce dried extract (36.2 g). A portion of dried extract (10 g) was fractionated with *n*-hexane (601.0 mg), DCM (150.5 mg), EtOAc (3453.8 mg), and residue (3029.7 mg). The *n*-hexane fraction (601.0 mg) was subjected to flash silica column chromatography (2-cm diameter, 30-cm length) and elution starting with *n*-hexane, transitioning to *n*-hexane (0.2 L), *n*-hexane:EtOAc (9.5:0.5, 0.5 L), *n*-hexane:EtOAc (9:1, 0.1 L), *n*-hexane:EtOAc (8.5:1.5, 0.4 L), *n*-hexane:EtOAc (8:2, 0.3 L), *n*-hexane:EtOAc (7.5:2.5, 0.2 L), *n*-hexane:EtOAc (5:5, 0.1 L), *n*-hexane:EtOAc (2.5:7.5, 0.1 L), EtOAc (100 L), EtOAc:ACN (5:5, 0.1 L), ACN (0.1 L), ACN:MeOH:H₂O (9:0.5:0.5, 0.1 L), and ACN:MeOH:H₂O (8:1:1, 0.1 L) to produce 110 fractions. TLC analysis of the fractions allowed isolation of crystals of compound **6** (4.1 mg) from fractions 16 and 17, and crystals of compound **3** (51.0 mg) from fractions 22 to 27. Fractions 39 to 42 were pooled and subjected to silica gel column chromatography (0.5-cm diameter, 6-cm length) and elution with EtOAc produced compound **7** (3.1 mg).

Extraction and Constituent Isolation of *Usnea misaminensis*

Dried powder (200 g) was stirred with MeOH for 48 hours at room temperature and then filtered and the supernatant vacuum dried to produce crude extract (16.6 g). Liquid-liquid fractionation of the rhizome extract produced *n*-hexane (1.28

g), DCM (3.26 g), and EtOAc (4.81 g), fractions along with a residue (1.45 g). The *n*-hexane fraction (0.2 g) was subjected to a short C₁₈ column (2 × 4 cm) and eluted with EtOAc. To this EtOAc fraction was added dropwise water and the resulting precipitate was collected, vacuum dried, and redissolved in DCM (2 mL) and left allowing the formation of yellow crystals of usnic acid **8** (60.5 mg). The DCM fraction (1 g) was subjected to short silica gel column chromatography (2 × 15 cm) and elution with *n*-hexane gave salazinic acid **9** (650 mg). The EtOAc fraction (0.5 g) was prepared for HPLC analysis by filtration through a short C₁₈ column (5 mL) and elution with DCM (100 mL) and MeOH:ACN (1:1, 100 mL) and concentrated to 25 mL. Preparative HPLC was employed with a gradient elution from 80% into 50% solvent A (0.1% TFA in H₂O) within 50 minutes with solvent B (0.1% TFA in ACN). The fraction collected at *t*_R 25 minutes was allowed to stand and needle crystals formed, identified as salazinic acid **9** (44.1 mg) by X-ray crystallographic analysis.

Crystallographic Data of Compound 8 (Usnic Acid)

Crystal data. Compound **8**. C₁₈H₁₂O₁₀•H₂O, *M* = 406.30, *T* = 150K, monoclinic, space group *P*2₁/*c*, *Z* = 4, *a* = 21.5208(11), *b* = 4.09693(14), *c* = 19.0128(7)Å, β = 103.744(5)°, *V* = 1628.33(12) Å³, *D*_x = 1.657 g/cm³, Cu *K*α radiation, λ = 1.54180 Å, 15 785 reflections measured (2θ = 4–140°) merged to 3 046 unique data, *R* = 0.043 [for 2547 data with *I* > 2σ(*I*)], *R*_w = 0.104 [all data], *S* = 1.02. Structure determination of compound **8**. Images were measured on an Agilent SuperNova diffractometer (Cu *K*α radiation, mirror monochromator, λ = 1.54180 Å) and data extracted using the CrysAlis PRO package.²² Structure solution was by direct methods (SIR92).²³ The structure was refined using the CRYSTALS program package.²⁴ Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1567131). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Crystallographic Data of Compound 9 (Salazinic Acid)

Crystal data. Compound **9**. C₁₈H₁₆O₇, *M* = 344.32, *T* = 200 K, orthorhombic, space group *P*2₁2₁2₁, *Z* = 8, *a* = 8.0273(1), *b* = 18.9068(3), *c* = 20.2525(3)Å, *V* = 3073.73(8)Å³, *D*_x = 1.488 g/cm³, Mo *K*α radiation, λ = 0.71073 Å, 43 467 reflections measured (2θ = 6–60°) merged to 5 024 unique data, *R* = 0.034 (for 4 436 data with *I* > 2σ(*I*)), *R*_w = 0.087 (all data), *S* = 0.99. Structure determination of compound **9**. Images were measured on a Nonius KappaCCD diffractometer (Mo

*K*α radiation, graphite monochromator, λ = 0.71073 Å) and data extracted using the DENZO package.²⁵ Structure solution was by direct methods (SIR92).²³ The structure was refined using the CRYSTALS program package.²⁴ Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1567130).

Extraction and Constituent Isolation of *Calymperes schmidtii*

A suspension of powdered of *C. schmidtii* whole plant (20 g) in MeOH (1 L) was stirred for 24 hours, filtered, and the filtrate concentrated to produce a dark green sticky semisolid (1.1 g). The crude extract in MeOH (100 mL) was liquid-liquid partitioned with *n*-hexane and both fractions were separately concentrated to produce an *n*-hexane fraction (360 mg) and a MeOH fraction (769 mg). A portion of the methanol fraction (250 mg) was applied into a short C₁₈ silica column (1.5 × 5 cm) and was then eluted with MeOH (50 mL) to give a solution, which was concentrated to 20 mL and filtered through a HPLC sample filter (0.45 μm). Analysis by HPLC using a gradient elution from 100% of solvent A (0.1% TFA in H₂O) to 0% of solvent A within 80 minutes (solvent B, 0.1% TFA in ACN) separated compounds **10** and **11** at retention times 39 and 45 minutes, respectively. Both fractions were vacuum dried to give pale yellow solids, methyl 2,4-dihydroxy-3,6-dimethyl-benzoate **10**, and methyl 2,4-dihydroxy-6-methylbenzoate **11**.

Bioactivity Testing

Antimalarial activity was determined against *Plasmodium falciparum* K1 based on the microculture radioisotope technique.²⁶ Cytotoxicity was tested against KB-oral cancer cell, lung cancer cell NCI-H187, breast cancer cell MCF7, and vero cells based on the resazurin microplate assay. The anti-TB assay utilized the *Mycobacterium tuberculosis* H37Ra strain using a green fluorescent protein microplate assay. Antiviral activity was tested against herpes simplex virus type 1 using a green fluorescent protein assay.²⁷

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

References

- Nugraha AS, Keller PA. Revealing indigenous Indonesian traditional medicine: anti-infective agents. *Nat Prod Commun.* 2011;6(12):1953-1966.
- Nugraha AS, Haritakun R, Keller PA. Constituents of the Indonesian Epiphytic Medicinal Plant *Drynaria rigidula*. *Nat Prod Commun.* 2013;8(6):703-705.
- Syamsuhidayat SS, Hutapea JR. *Inventaris tanaman obat Indonesia*. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan Republik Indonesia; 1991: Volume 1. 239-240.
- Asakawa Y. Liverworts-potential source of medicinal compounds. *Curr Pharm Des.* 2008;14(29):3067-3088.
- Chandra S, Chandra D, Barh A, Pankaj, Pandey RK, Sharma IP. Bryophytes: Hoard of remedies, an ethno-medicinal review. *J Tradit Complement Med.* 2017;7(1):94-98.
- Asakawa Y. Biologically active compounds from bryophytes. *Pure Appl Chem.* 2007;79(4):557-580.
- Rao GV, Rao PS. A new flavonol glycoside from the flowers of *Crotalaria verrucosa*. *Fitoterapia.* 1985;56(3):175-177.
- Baldé AM, Apers S, De Bruyne TE, et al. Steroids from *Harrisonia abyssinica*. *Planta Med.* 2000;66(1):67-69.
- He R, Zou B, Fang H, Zhang Y, Wu Y. Free radical scavenging activities of b-Sitosterol extracted from *Abies yuanbaoshanensis*. *Asian J Chem.* 2013;25(6):3507-3508.
- Atalay Halici FF, Mavi A, Cakir A, et al. Antioxidant phenolics from *Lobaria pulmonaria* (L.) Hoffm. and *Usnea longissima* Ach. lichen species. *Turk J Chem.* 2011;35(4):647-661.
- Mendiondo ME, Coussio JD. Chemical investigation of Argentine lichens. II. Usnic, norstictic, and salazinic acids from *Usnea densirostra* and *U. angulata*. *Phytochemistry.* 1972;11(1):424.
- Correche ER, Carrasco M, Escudero ME, et al. Study of the cytotoxic and antimicrobial activities of usnic acid and derivatives. *Fitoterapia.* 1998;69(6):493-501.
- Candan M, Yilmaz M, Tay T, Erdem M, Türk Aysen Özdemir, Türk AO. Antimicrobial activity of extracts of the lichen *Parmelia sulcata* and its Salazinic acid constituent. *Z Naturforsch C.* 2007;62(7-8):619-621.
- Bauer J, Waltenberger B, Noha SM, et al. Discovery of depsides and depsidones from lichen as potent inhibitors of microsomal prostaglandin E2 synthase-1 using pharmacophore models. *Chem Med Chem.* 2012;7(12):2077-2081.
- Bourgeois G, Suire C, Vivas N, Vitry C. Atraric acid, a marker for epiphytic lichens in the wood used in cooperage: identification and quantification by GC/MS/MS. *Analisis.* 1999;27(3):281-283.
- Musharraf SG, Kanwal N, Thadhani VM, Choudhary MI. Rapid identification of Lichen compounds based on the structure-fragmentation relationship using ESI-MS/MS analysis. *Anal Methods.* 2015;7(15):6066-6076.
- Roell D, Baniahmad A. The natural compounds atraric acid and *N*-butylbenzene-sulfonamide as antagonists of the human androgen receptor and inhibitors of prostate cancer cell growth. *Mol Cell Endocrinol.* 2011;332(1-2):1-8.
- Suárez AI, Mancebo M, Delle Monache F, et al. A new indole-alkaloid and a new phenolic-glycoside with cytotoxic activity from *Strychnos fendleri*. *Nat Prod Res.* 2016;30(4):399-405.
- Chumkaew P, Kato S, Chantrapromma K. New cytotoxic steroids from the fruits of *Syzygium siamense*. *J Asian Nat Prod Res.* 2010;12(5):424-428.
- Zuo S-ting, Wang L-ping, Zhang Y, et al. Usnic acid induces apoptosis via an ROS-dependent mitochondrial pathway in human breast cancer cells in vitro and in vivo. *RSC Adv.* 2015;5(1):153-162.
- Honda NK, Pavan FR, Coelho RG, et al. Antimycobacterial activity of Lichen substances. *Phytomedicine.* 2010;17(5):328-332.
- Technologies A; 2013. CrysAlis PRO, Version 1.171.37.21t. Yarnton, Oxfordshire, England.
- Altomare A, Cascarano G, Giacovazzo C, et al. SIR 92 – a program for automatic solution of crystal structures by direct methods. *J Appl Crystallogr.* 1994;27(3):435-436.
- Betteridge PW, Carruthers JR, Cooper RI, Prout K, Watkin DJ. CRYSTALS version 12: software for guided crystal structure analysis. *J Appl Crystallogr.* 2003;36(6):1487.
- Otwinowski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. In: Sweet RM, ed. *Methods in Enzymology, Macromolecular Crystallography, Part A, Volume 276*. New York, NY: Elsevier; 1997:307-326.
- Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob Agents Chemother.* 1979;16(6):710-718.
- Haritakun R, Sappan M, Suvannakad R, Tasanathai K, Isaka M. An antimycobacterial cyclodepsipeptide from the entomopathogenic fungus *Ophiocordyceps communis* BCC 16475. *J Nat Prod.* 2010;73(1):75-78.