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Usnea in West Java: a potential source of bioactive secondary metabolites

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Abstract

Lichen is a symbiotic organism consisting of algae (photobionts) and fungi (mycobiont). In Indonesia, *Usnea* is the only genus of lichen that has been widely recognized and used by the local community for traditional medicine. It is locally known as kayu angin, rusuk angin, or janggot Kai. Furthermore, it produces lichen acid and other compounds as secondary metabolites that need investigation. Therefore, this study aimed to identify species and determine the secondary metabolites of *Usnea* in West Java. There were 13 species of Usnea identified, with *U. rubrotincta, U. mutabilis, and U. barbata* being new to West Java. The extraction of *U. esperantiana* has the highest number of lichen acids, with 20 different types identified. This study showed that *Usnea* contains lichen acid, flavonoid, and saponin compounds. The secondary metabolites are a potential source of natural products for bioprospecting.

Keywords: bioprospecting, lichen acid, new record, secondary metabolite, *Usnea*

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Introduction

Lichen is a symbiotic organism consisting of algae (photobionts) and fungi (mycobiont). (Dobson, 2011). Lichen produces a variety of bioactive substances that serve as defense mechanisms against physical stress and biological attack and maintain the symbiotic relationship's stability (Crittenden & Porter, 1991; Muggia et al., 2009; Huneck, 2001). Lichen acid is a secondary metabolite chemical produced by lichen. It is formed by mycobionts and accumulates on the extracellular surface of fungal hyphae. Each species of lichen produces a unique combination of secondary metabolites. However, they have fixed characteristics for each species, which is necessary for species identification (Hale, 1969). For example, Usnea is a lichen belonging to the Parmeliaceae family and comprises 600 species worldwide (Ohmura, 2001).

Lichens produce secondary metabolites such as amino acid derivatives, pulvinic acids, peptides, sugars, alcohols, terpenoids, steroids, aliphatic acids, monocyclic phenols, anthraquinones, xanthones, usnic acids, and other compounds (Huneck, 1999). The content of lichen acid in the genus has been widely known for its role, especially in the pharmaceutical field, having activity as an anticancer (Muggia et al., 2009), antibiotics (Kosanic et al., 2019), antiproliferative (Mitrović et al., 2011), and antiviral (Sepahvand et al., 2021). However, the content of other lichen acids has not been widely used.

The information on the content of secondary metabolites other than usnic acid is needed from each species to maximize the potential in medicine and other fields. In Indonesia, *Usnea* is found in mountainous areas at more than 800 masl, with high humidity, and on old tree trunks (Heyne, 1987). Previous studies reported the type of lichen acid in several species from the Priangan (Noer, 2013) and Bogor regions (Jannah, 2012). However, there is no data on Usnea from other parts of West Java. Therefore, this study aims to identify the species that grows in the West Java forest and determine the secondary metabolites. This data is critical for updating the Indonesian Herbal Pharmacopoeia, which guides the production of traditional medicines, phytopharmaceuticals, and standardized herbal medications.

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Methods

The samples were collected from the forest around the West Java region (Orchid forest, Lembang and Langkaplancar, Pangandaran). The identification of *Usnea* was based on morphological, anatomical, and microchemical characteristics. Furthermore, microchemistry observations were conducted by slicing lichen thallus and observing the medulla's color change after dripping with Potassium hydroxide Platzchen and Calcium Hypochlorite.

Observation of the type of lichen acid based on microcrystals was performed by extracting the thallus using acetone solution, then left until white crystals were formed. The crystals were then dripped with G.A.An reagent. (glycerin: alcohol: aniline = 1:2:2) and G.A.OT. (glycerin: alcohol: O-toluidine = 1:2:2) before microscopic observation. The crystal was also observed using G.A.W reagents (glycerin: alcohol: water = 1:1:1) and G.E. (glycerin: acetic acid = 1:3) by heating and then observed under a microscope (Jannah, 2020). Additionally, the shape was determined under a lowpower microscope (100x), and the crystal was identified by comparison with photographs (Huneck, 1999). The extract was made by boiling 2.5 g of Usnea and adding 100 ml of water as a solvent.

A qualitative test was performed to determine the presence of alkaloids, flavonoids, tannins, and saponins following Oktari et al. (2014). The flavonoid test was carried out by dissolving 1 ml of extract in 1 ml of 95% ethanol. An amount of 0.1 g of Magnesium powder and 10 drops of concentrated HCl were added and vigorously shaken. A positive test resulted in forming a red, yellow, or orange color. To perform the tannin test, 1 ml of the extract was diluted with 2 mL of distilled water, followed by 3 drops of $FeCl_3$ solution. A shift in the hue of the solution color to blue-black or green-black suggests a positive result.

Results

The genus *Usnea* is separated from other genera of Parmeliaceae by the fruticose thallus having a central axis and usnic acid in the cortex. The different species can be distinguished based on thallus morphology, apothecia, growth type, branching type, the base of the thallus, medulla type, pigment around the medulla and cortex, type and color of the central axis, apothecia, the most dominant vegetative organs, pseudocyphellae, foveoles, fibrils, isidiomorph, papillae, microchemical test, and substance content. The 13 species obtained were divided into three subgenera: *Usnea*, *Eumitria*, and *Dolichousnea*. The subgenus *Usnea* consists of *U. rubrotincta*, *U. fragilescens*, *U. intermedia*, *U. esperantiana*, *U. hesperina*, U. *mutabilis*, *U. barbata*, and *U. flammea*. Meanwhile, the subgenus *Dolichousnea* consists of *U. longissima*, *U. hirta*, and *U. dasypoga*, and the subgenus *Eumitria*, namely *U. pectinata*, and *U. baileyi*. The diagnostic character of the *Dolichousnea* consists of annular-pseudocyphellae between segments. The *Eumitria* and *Usnea* have a fistulose-type central and a solid-type central axis, respectively (Fig. 1)

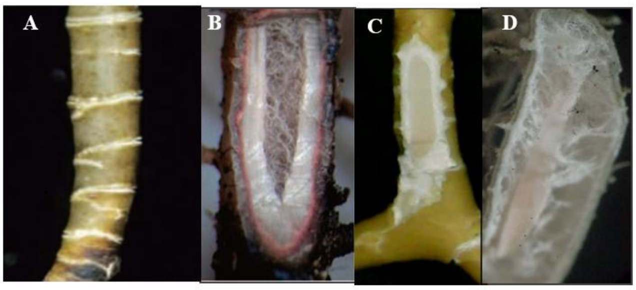


Figure 1. The diagnostic character between subgenus, A. annular-pseudocyphellae (*Dolichousnea*), B. Fistulose axis (*Eumitria*), C-D. Solid Axis (*Usnea*).

Three species, *U. rubrotincta, U. mutabilis, and U. barbata*, are new records for the genus Usnea in West Java (Noer, 2013; Kusmoro, 2018; Jannah, 2020). The detailed description of each species is as follows.

***Usnea rubrotincta* Stirt.**

Usnea rubrotincta Stirt., Scott. Natur. 6: 103, 1881 (*Usnea rubescens* Stirt., *Usnea rubescens* subsp. *aberrans* Asahina, *Usnea rubescens* subsp. *rubescens* Stirt., *Usnea rubescens* var. *anaemica* Asahina, *Usnea rubescens* var. *areolata* Motyka, *Usnea rubescens* var. *rubescens* Stirt., *Usnea rubescens* var. *rubrotincta* (Stirt.) Motyka)

The features of this species are green thallus, suspended, anisotomic-dichotomous, base dark brown to black, apothecia absent, fibril short and irregularly distributed, papillae, and pseudocyphellae irregularly distributed on the thallus, rounded soralia, red pigment cortex, solid central axis, and compact medulla. The chemical test in the medulla is K+red, C-; and it contains 4-0 metylphysodic, fumarprotocetraric, usnic, atranorin, obtusatic, imbricaric, protocetraric, physodalic, and diffractaic acids (Fig. 2).

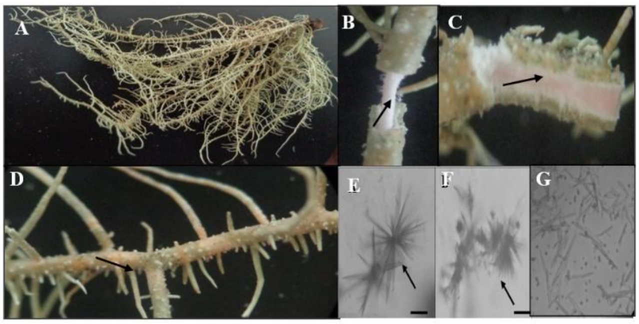


Figure 2. Morphological structure of *U. rubrorincta*: A. Morphology of thallus, B. Central axis, C. Medulla compact, D. Fibril, E. Usnic acid, F. Fumarprotocetraric acid, G. Diffractaic acid.

The species resembles *U. rubicunda* but differs in branches, soralia, and fibril (Ohmura, 2001). Furthermore, *U. rubrotincta* has a unique character in the number of fibrils, lateral branches, and red pigment in the cortex.

According to Ohmura (2012), the species contains salazinic and stictic acid as the main content. However, the species has different chemotypes such as 4-0 methylphysodic, fumarprotocetraric, usnic, atranorin, obtusatic, imbricaric, protocetraric, physodalic, and diffractaic acid.

***Usnea barbata* (L.) F.H. Wigg.**

(*Lichen barbatus* L., *Usnea dasopoga* f. *scabrata* (Nyl.) Arnold, *Usnea scabiosa* Motyka, *Usnea scabrata* Nyl)

The features of this species are green thallus, pendent, isotomic-dichotomous, brown or brownish-black branches, apothecia absent, numerous fibril, irregular branches, papillae with infrequent isidia, rounded soralia, solid central axis, and loose medulla. The chemical test in the medulla are K+yellow to red, C-, KC+yellow; they contain usnic acid and salazinic acid (Fig. 3).

***Usnea mutabilis* Stirton**

The features are yellowish-green thallus, erect-shrubby, anisotomic-dichotomous, dark brown base, apothecia absent, infrequent fibril, rounded soralia, numerous isidia, solid central axis, dense to compact medulla with brownish-pink pigment. The chemical tests in the medulla are K+yellow to red, C-, KC+yellow to red, containing usnic and norstictic acid (Fig. 4).

This species resembles *U. rubicunda* based on morphological and chemical analysis, and it contains usnic, norstictic, menegazziaic, and stictic acids. In some countries such as Taiwan, *U. mutabilis* is absent. However, it is widely distributed in temperate regions of Japan, Europe, and North America (Clerc, 1998; Ohmura, 2001)

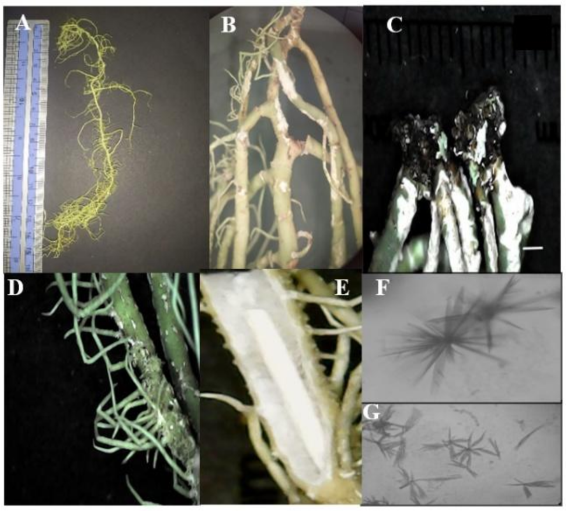


Figure 3. Morphological structure of *U. barbata*: A. Morphology of thallus, B. Branch type, C. Base, D. Fibril, E. Medulla, F. Usnic acid, G. Salazinic acid.

Figure 4. Morphological structure of *U. mutabilis*: A. Morphology of thallus, B. Base, C. Medulla, D. Soralia, E. norstictic acid, F.Usnic acid.

Discussion

The lichen acid contained in *Usnea* spp.

The Lembang and Pangandaran (West Java) forests have cool air. They are geographically located at an altitude of 1,500 masl, with an average annual rainfall of 2,700 mm and an air temperature of 12-29 °C (Dinas Pariwisata dan Kebudayaan Jawa Barat, 2020). These environmental conditions are suitable for lichen growth habitats, especially *Usnea* (Josefsson et al., 2005). Lichen prefers a moist environment that promotes growth and the production of the lichen's unique secondary metabolite.

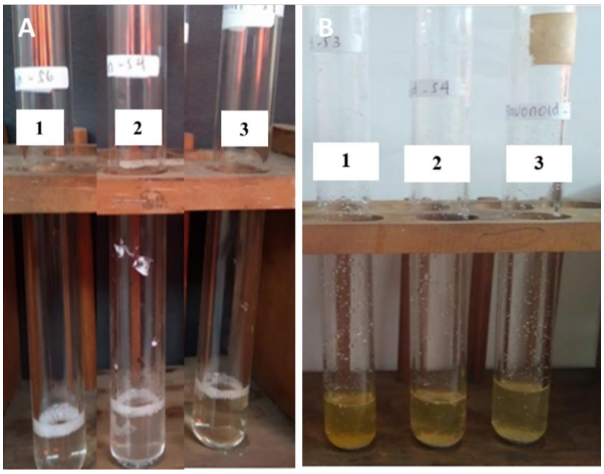


Figure 5. Positive test result saponin showing bubbles (A) and flavonoid showing colour change (B). 1. U. rubrotincta, 2. U. mutabilis, 3. U. barbata

The compound components react with specific tests using chemicals to give a color reaction used in species identification. The phytochemical screening showed that all species of *Usnea* contain flavonoid and saponin compounds, but no tannins were detected (Fig. 5). The dominant lichen acid content in the species is usnic acid (Cansaran et al. 2006; Bessadóttir 2014). Furthermore, antibiotics, anticancer, antimutagenic, analgesic, anti-inflammatory, antiviral, allergic, and antioxidant properties are found in the species obtained from West Java (Table 1).

Usnea can be processed into herbal medicine by boiling the plant with water, ginger, turmeric, and other components. *Usnea baileyi* is used as a mixture for lung disease, while *U. blepharea* treats leprosy, cough, and skin disease (Maulidiyah, 2011). Subsequently, *Usnea barbata* has been used in cosmetic products for its antimicrobial and antifungal properties as a preservative and deodorant. Other uses for traditional Usnea species have been recorded by residents of Bogor and Jakarta (Jannah, 2020).

Table 1. The secondary metabolites of *Usnea* from West Java

Species	Lichen Acid	Phytochemical
U. intermedia	Didymic acid, hiasic acidv, acetoxyhopan, virensic acid, fumarprotocetraric acid, usnic acid, friedelin,	Flavonoid, Saponin

	psoromic acid, lobodirin, caperatic acid, grayanic acid, leucotylin, alectorialic acid, barbatic acid, 4-O-methylphysodic acid, umbilicaric acid, acetylportentol, and salazinic acid	
U. esperantiana	galapagin, hypoprotocetraric acid, methyl 3,5-dichlorolecanoratem, usnic acid, planaic acid, retigeric acid, leucotylin, hiasic acid, roccellaric acid, 4-O-methylphysodic acid, imbricaric acid, lobodirin, alectorialic acid, lichesterinic acid, umbilicaric acid, acetylportentol, norstictic acid, salazinic acid, psoromic acid, merochlorophaeic acid, and hypoprotocetraric acid	Flavonoid, Saponin
U. hesperina	Usnic acid, didymic acid, chloroatranorin, norstictic acid, hypoprotocetraric acid, atranorin, ursolic acid, ramalinolic acid, sekikaic acid, arthothelin, imbricaric acid, protocetraric acid, gangaleoidin, salazinic acid, 4-O metylphysodic acid, lobodirin, barbatic acid, confluentic acid, and hiascic acid	Flavonoid, Saponin
U. pectinata	norstictic acid, pseudoplacodiolic acid, usnic acid, imbricaric acid, perlatolic acid, psoromic acid, didymic acid, α-alectoronic acid, α-collatolic acid, 4-O-methylphysodic acid, gangaleoidin, acetylportentol, and hiascic acid	Flavonoid, Saponin
U. fragilescens	usnic acid, hiascic acid, galapagin, virensic acid, placodiolic acid, lichesterinic acid, norstictic acid, gangaleoidin, atranorin, physodalic acid, planaic acid, confluentic acid, protocetraric acid, stictic acid, barbatic acid, psoromic acid, and lobaric acid	Flavonoid, Saponin
U. baileyi	pseudoplacodiolic acid, usnic acid, virensic acid, isousnic acid, thamnolic acid, α-acetylsalazinic acid, protocetraric acid, diffractac acid, atranorin, obtusatic acid, friedelin, barbatic acid, norstictic acid, 4-O-methylphysodic acid, lichesterinic acid, caperatic acid, and imbricaric acid	Flavonoid, Saponin
U. dasypoga	salazinic acid, and usnic acid	Flavonoid, Saponin
U. barbata	usnic acid, and salazinic acid	Flavonoid, Saponin
U. mutabilis	norstictic acid, and usnic acid	Flavonoid, Saponin
U. rubrotincta	4-0 metylphysodic acid, fumarprotocetraric acid, usnic acid, atranorin, obtusatic acid, imbricaric acid, protocetraric acid, physodalic acid, diffractaic acid, and stictic acid	Flavonoid, Saponin
U. flammea	Stictic acid, norstictic acid, and lobaric acid	Flavonoid, Saponin
U. hirta	Alectronic acid, divaricatic acid, lecanoric acid, norstictic acid, salazinic acid, usnic acid, and perlatoric acid	Flavonoid, Saponin
U. longissima	Atranorin, alectronic acid, barbatic acid, divaricatic acid, norstictic acid, usnic acid, and perlatoric acid	Flavonoid, Saponin

Usnea is used in traditional medicine, food coloring, and seasoning in India. Meanwhile, *Usnea longissima* has been widely administered as an expectorant, treating ulcers and fractures. In the Unani literature, it is mentioned that *Usnea* spp. has been used as astringent, analgesic, cardiotonic, solvent, and stomachic (Raut et al., 2011). In some countries, the content of secondary metabolites from lichen acid is quite attractive as an alternative treatment and used in the pharmaceutical industry (Huneck 1999; Oksanen 2006). The biological potential of lichen, especially *Usnea*, is enormous; however, the pharmaceutical and other industries have neglected its existence. This is due to the slow-growing nature of the thallus and the difficulty in the cultivation process (Behera et al., 2003, 2004).

Most of the *Usnea* in the West Java area contains usnic, salazinic, and norstic acids. Lichen acid has been reported to have medicinal potential (Cobanoglu et al. 2010; Crawford 2015). Furthermore, *Usnea* secondary metabolites exhibit antimicrobial, antioxidant, anti-inflammatory, cytotoxic, analgesic, antipyretic, and antiviral properties (Rancovic, 2014) (Table 2). Some potential examples of lichen acid are described below (Table 2).

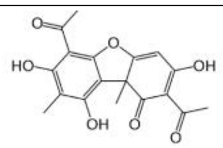
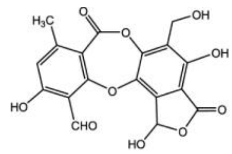
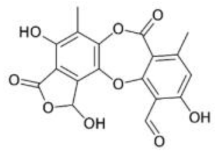
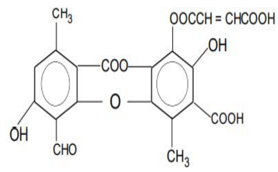
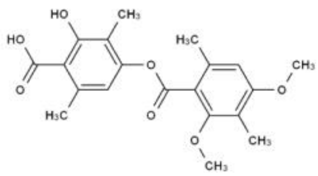
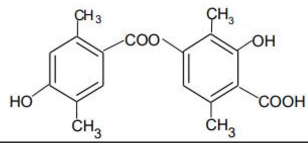
The presence of a stable foam indicated positive results for saponin compounds for 5 min after vigorous shaking. The results showed that all *Usnea* species were positive for saponins. According to Robinson (1995), the formation of a stable foam after shaking indicates a high saponin content in the sample. The saponin content in the Usnea species can bind cholesterol and external wound therapy to block blood flow on the skin. It also has biological activity as a natural surfactant, antibacterial, decreases blood sugar levels, and prevents blood clots (Minarno, 2016).

The *Usnea* obtained were also contain flavonoids, marked by yellow to orange colors. Flavonoids are the largest group of phenolic compounds found in nature. Its compounds have antioxidant properties, and research into Usnea's antioxidant potential is currently in the laboratory testing stage (Agustina, 2016).

Flavonoids are antioxidants that can protect the body from damage caused by free radicals. The most significant concern of free radicals is premature aging (Harrijanto, 2018). Therefore, the flavonoid compounds in *Usnea* can be used in beauty products to prevent early aging. *Usnea* also contains saponins for healing internal diseases. The antibacterial and natural surfactants can also act as external drugs for itchy skin (Nurzaman et al*.,* 2018).

According to the study results, *Usnea* contains antibacterial, antifungal, antiviral, anticancer, antioxidant, anti-inflammatory, antiprotozoal, analgesic, and antipyretic bioactive compounds. Therefore, this species have potential to be developed into medicinal raw materials.

Table 2. The Structure of secondary metabolites in the West Java *Usnea* species

No	Secondary metabolites in *Usnea*	Structure	Activity	Reference
1.	Usnic acid		Antiviral, antitumour, antioxidant, antibacterial, antifungal, antipyretic, analgetic, anti-inflammatory, hepatotoxic, antiviral	Paudel et al. (2010); Bazin et al. (2008); Burlando et al. (2009)
2	salazinic acid		Antibacterial activity, antioxidant acitvity	Sultana (2011)
3.	Norstictic acid		Antimicrobial, antioxidant, anticancer	Tay et al. (2004); Honda et al. (2010); Rankovic et al. (2014)
4.	Fumaroprotocetraric acid		Antimicrobial, antifungal	Rankovic et al. (2008); Yilmaz et al. (2004)
5.	Diffractaic acid		Analgetic, antiproliferative, antioxidant, antipyretic	Brisdelli et al. (2013); Atalay et al. (2011)
6.	Barbatic acid		Antimicrobial activity, antiproliferative activity	Martins et. al. (2010)

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