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Lichen diversity in geothermal area of Kamojang, Bandung, West Java, Indonesia and its potential for medicines and dyes

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[2]Undergraduate of Biology Program Study Faculty of Mathematics and Natural Sciences, Padjadjaran University. Jl. Raya Bandung Sumedang Km. 21, Jatinangor, Sumedang 45363, West Java, Indonesia
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Abstract. *Kusmoro J, Noer IS, Jatnika MF, Permatasari RE, Partasasmita R. 2018. Lichen diversity in geothermal area of Kamojang, Bandung, West Java, Indonesia and its potential for medicines and dyes. Biodiversitas 19: 2335-2343.* The study of lichens diversity in Kamojang, West Java was conducted by survey in geothermal field area following the line transect 6 km along to the East, North West and south from the Power House of Geothermal Power Plant. The lichen samples were taken from bark, soil, and stone. Lichen identification was done by morphological, anatomy and chemical analysis. Dyes potency of *Parmotrema* and *Usnea* test using ammoniac fermentation was done in Plant Taxonomy Laboratory of Department Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran. The survey has successfully collected 133 species of lichens, belong to 62 genera and 17 families. *Parmeliaceae* was found as dominant groups, consisting of 33 species and other co-dominant groups are *Graphidaceae* and *Lobariaceae* with 24 species and 8 species, respectively. Most lichens in Kamojang geothermal area belong to Ascomycetes, only one Basidiomycetes such as Dictyonema sericeum (Sw.) which found at Kawah Manuk (Manuk crater) area. The rare species of lichens such as *Usnea longissima* Ach, was found at Pine forest in Arboretum 6 km south of Powerhouse of Kamojang geothermal. Chemical analysis and literature study for Lichenic acid contains was done and generally, atranorin, usnic acid, barbatic and lecanoric acid was found in lichens samples. Amoniac fermentation result showed that *Parmotrema tinctorum* produced brownish red, red and purple, which occurred within 1 week to 5 weeks after fermentation. While *Usnea* produced variety of brown color, which occurred within 5 days up to 4 weeks after fermentation. Lichen species containing some medical properties are *Bulbothrix, Cladonia* and *Usnea.* While lichens having dyes properties are *Hypogymnia, Lobaria, Peltigera, Usnea*, and *Parmotrema.*

Keywords: Dye, fermentation, Kamojang, lichen, lichenic acid, medicine

INTRODUCTION

Kamojang Geothermal Area, which is recognized as mountain tropical rain forest, is one of Natural Conservation and Tourism forest in West Java. It is well known as hilly area with beautiful view. It has crater and hot spring water for medical purposes and the source of Geothermal Energy. Kamojang is also natural forest with high diversity of plant, mosses, and lichens. Lichens have the symbiotic phenotype with nutritious fungi and are also associated with algae (Noer 2013; Ardelean et al. 2015; Balabanova et al. 2014; Onuț-Brännström 2017). These are an outstanding group, exploiting a wide range of habitats throughout the world and dominating about 8% of terrestrial ecosystems. They have a varied chemical contains, which are useful as medicines (Noer et al. 2013 Onuț-Brännström 2017). Lichens contain secondary compounds that are abundant in most lichen thalli. Different lichens produce a wide variety of metabolite compounds, most of which are unique to lichens (Noer et al. 2006). It is suspected that almost 50% of species of lichens have antibiotic properties (Vartia 1973; Malhotra et al. 2007; Crawford 2015). Lichen compounds have been found to act as anti-tumor agents, antibiotics, and anti-inflammatories (Bayir et al. 2006; Rezanka and Dembitsky 2006; Bessadóttir 2014). Some of the most widely studied lichen compounds are usnic acid, vulpinic acid, atranorin, and protolichesterinic acid (Asahina and Shibata 1954; Fazio et al. 2007; Crawford 2015; Salgado 2018). Usnic acid is found in large quantities in *Usnea* spp., as well as in several other lichen genera.

It is well known that a long time ago lichens have been widely used by people such as for medicine, pollution bioindicator, perfume, decoration, and dye (Ingolfsdottir et al. 2002; Kaasalainen 2012; Vicol 2016; Tarasova et al. 2017). Lichens as natural dye sources have been used since long ago. The purple color (orchil) was firstly reported from *Roccella* spp. through ammoniac fermentation. The purple color of *Roccella* had historic importance as the "Royal Purple" in Europe before 19th century and was not been used anymore when synthetic dye substance was found.

Genus *Parmotrema* is foliose lichen that belongs to Parmeliaceae group. The genus characteristic is the absence of reticular maculate and pseudocyphella. An erhizinate marginal zone of the lower surface is more than 1 mm

(Noer 2013). Several *Parmotrema* species from Himalayas can produce colors with the help of ammonia fermentation method (Shukla et al. 2014). Livelihoods of natural dyes from lichen *Parmotrema* are very interesting and will be important in the use of dyes that are environmentally friendly in industry in order to reduce the pollution of water/river by dyes from industry.

The diversity of lichen in this area has been reported by Noer et al. (2006) and Noer and Rani (2007), but only one species has been used as medicine i.e. *Usnea barbata*, no mentioning about the species has potential for dyes. The continuous survey was done in this area to collect some species, which has potential use for medicine and dyes. Our purpose from the study were: (i) to assess the diversity and distribution of lichens in Kamojang Geothermal Area, (ii) to indicate several lichens, which could potentially be used as medicine and dyes, (iii) to analyse the lichens acids (secondary metabolites) in potential lichen genus for medicine and dyes, (iv) to monitor color produced by *Parmotrema* and *Usnea* using ammonia fermentation.

MATERIALS AND METHODS

Study area

Kamojang area belonging to mountain tropical forest is located at Laksana village, Ibun Sub-district, Bandung District, West Java, approximately 45 km southeast of Bandung City. The research area is located on latitude of 7°8′23.13″ S 107°47′10.36″ E to 7 °00’00” S 107°00’00” E with altitude 1300-1700 above sea level (Figure 1). This exploration was held from April 2016 to August 2017

The microclimate was recorded that the range of temperature ranging from 18^0 C to 26,26 ^{0} C, while the humidity was from 56.13 % to 78% and light intensity had 2387 lux to 6748 lux.

Sampling

The research material was lichen specimens from Kamojang geothermal area. The lichen collection in the study area was taken with survey method at 6 km transects towards four cardinal points (N, E, W, S) from the Power House of Geothermal Power Plant as the center point. The sample was taken from bark along the road and stone in the study area. Dry specimens were stored in paper bags and wet specimens were needed to be dried first to prevent molding. The specimens were then moistened, pressed flatly between boards, dried and placed on paper packets (3 x 4 inches) or on small boxes.

Lichen identification

Identification of lichens was conducted by: (i) Determine the growth form; foliose, squamulose, crustose or fructocose. These characters are important in separating

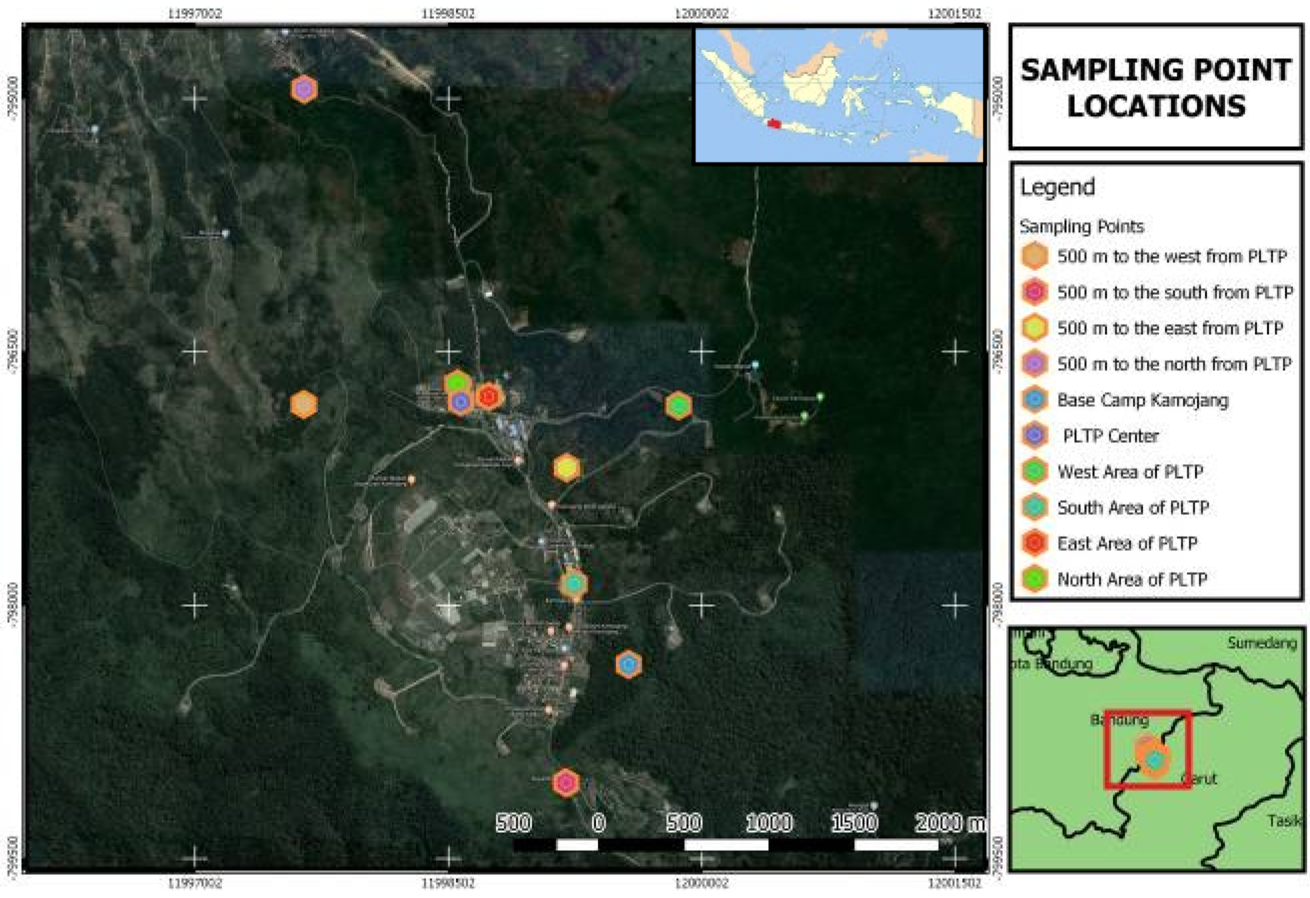

Figure 1. Maps of the research in Kamojang geothermal area, Bandung, West Java, Indonesia

genera. (ii) Checking the presence of soredia or isidia with a hand lens. These are by far the two most important characters used in identifying lichens at the species level and must be recognized without any doubt. Presence of cilia and the condition of lower side should also be noted. (iii) Chemical test in lichen was used i.e color test and crystal test.

A color test was made simply by applying a drop of reagent on the thallus surface or exposed medulla. If the test is positive, there will be a rapid color change, usually red or yellow; if it is negative nothing will happen. Three different reagents were used, i.e., calcium hypochlorite (bleaching powder, abbreviated C), potassium hydroxide (caustic lye abbreviated K or KOH) and paraphenylene-diamine (P). The K+ yellow test caused by atronin.

In a crystal test, the acid was dissolved from small fragments of thallus with acetone, and the remainder of crude residue was recrystallized from various reagents on a microscope slide. The reagents in common use are abbreviated as follows and mixed in the volume ratios including: (i) G.E. (glycerin-acetic acid, 3: 1), (ii) G.A.W. (glycerin-95% alcohol-water, 1: 1: 1), (iii) G.A. o-T (glycerin-alcohol-o-toluidine, 2: 2: 1), (iii) G.A.An (glycerine-alcohol-aniline, 2: 2: 1), (iv) G.A.Q. (glycerine-alcohol-quinoline, 2: 2: 1).

Fragment of the lichen thallus was heaped in the center of microscope slide, and drops of acetone was added several times. After the acetone evaporates, there should be whitish or yellowish powdery ring of residue. The thallus fragments were carefully brushed away, a small drop of reagents put on a coverslip, and the coverslip placed over the residue. The slide is gently heated over an alcohol lamp, low Bunsen flame until bubbles just began to form. On cooling a few minutes, crystal began to form first around the undissolved residue, later at the perimeter of the coverslip. The shape and of the crystals were determined under a low-power microscope (100x), and the crystal identified by comparison with photographs.

Lichen dyes

For dyeing with lichens, three different methods were used depending to the desired color: (i) BWM-simply means “boil water method”. (ii) AM-“ammonia method” and (iii) POD stand for “Photo Oxidizing Dyes”. In a preliminary study, the ammonia method was used with the detailed method as follow: (i) The lichens *Parmotrema tinctorum* were collected from clean areas in Kamojang and then were air-dried and subjected for extra Ammoniac extraction. (ii) Six gram of thallus lichens samples were put into a glass mason jar with an airtight lid. To which 100 milliliter (ml) of 10 % ammonia (NH_3) and 100 ml distilled water was added. (iii) Close the lid and shake vigorously. Within minutes the color shift will arise. (iv) The jar then labeled with the date, location collected and the name of species. (v) The mixed solution was fermented in the dark for 4 weeks and allowed to stand for three months and every day the jar was shaken. (vi) The monitoring of color extracts was recorded during fermentation for each week.

RESULTS AND DISCUSSION

Lichen diversity

Generally, lichens in Kamojang Geothermal areas have a high diversity consisting of more than 133 species belong to 62 genera and 17 families. Families of Parmeliaceae found in this study was dominant consisting of 33 species and the co-dominant species such as Graphidaceae and Lobariaceae with 24 species and 8 species, respectively (Table 1).

Most lichen fungi obtained in this study belong to Ascomycetes, but the fan-shaped of this lichens is a basidiomycetous lichen with cyanobacterial photobionts. It is *Dictyonema sericeum* (Sw.) Berk (Figure 2), which was found at Kawah Manuk area. Dictyonema is a symbiosis between a basidiomycete fungus and *ascytonematoid* cyanobacteria, resulted in both a basidiolichen and a cyanolichen, which is a very rare combination.

The protected species as *Usnea longissima* Ach. could only found in cleanest area at pinus forest in Arboretum area which 6 km south of Kamojang Geothermal Power Plant (Figure 2). In an effort to determine the diversity of lichens in Kamojang and to determine the species of lichen that has been utilized as medicine and dyes by the local community we performed inventory, literature study and literature process with the local community.

Lichen biodiversity is often used to assess air quality and ecosystem health within non-urban environment. This survey, in 2017 as a result in Table 1, shown one hundred thirty-three (133) species lichens have been found in Kamojang Geothermal area. But the study has been done in 1983, 1987 and 1988 lichen was found only 53 species (Noer et al. 2013). Compare to those survey, lichen in Kamojang geothermal area more diversity although the Geothermal Power Plan has operation since 1983 and produced SO^2which has been known that doses 0.018 ppm of SO^2 will kill lichens (Pearson and Skye1965; State et al. 2010; Rubio-Salcedo et al. 2017). The amazement *Graphis kamojangense* was found on *Mangifera indica* and *Toena sureni* trees at 0.5 km west and 1.5 km south from Geothermal Power Plan.

The lichenic acid contained in genus of lichens

Components of lichens reacting with certain test chemicals may give color reactions, which could be used in the identification of a species (Figure 3). The phytochemical screening of *Usnea* showed that beard moss contains alkaloid, steroid, saponin, monoterpenoid, sesquiterpenoid, quinone, and polyphenol (Choudhary et al. 2005; Fazio et al. 2007; Güllüce et al. 2006). The lichenic acid was found dominantly in *Usnea* are usnic acid and Stictic acid (Cansaran et al. 2006; Bessadóttir 2014). Some lichenic acids was found in several genus from Kamojang Geothermal area such as alectoronic acid, atranorin acid, barbatic acid, baeomycesis acid, chloroatranorin acid, diffrataic acid, diploicin acid, divariatic acid, gyrophoric acid, grayanic acid, haematamnolic acid, isousnic acid, lecanoric acid, leucotylin acid, lobaric acid, obtusatic acid, physodalic acid, protocetaric acid, perlatolic acid, retigeric acid, rocellic acid, salazinic acid, stenosporic acid, stictic acid, thiopanic acid and usnic acid.

Table 1. Lichens distribution at Kamojang geothermal area, Bandung, Indonesia

Species	Reproduction	Reagent	Lichen acid
Acarospora sp.	Apothecia	-	Norstictic acid. rhizocarpic acid gyrophoric acids epanorin acids
Amandinea punctata (Hoffm) Coppins & Scheid	-	-	-
Bacidia sp.	-		No lichen substances
Buellia punctata (Hoffm) A. Massal	Soredia and isidia	-	-
Buellia sp.1	Soredia and isidia	-	-
Bulbothrix isidiza (Nyl.) Hale	Soredia and isidia	GAW	Barbatic acid, consalazinic acid, gyrophoric acid, lobaric acid, salazinic acid, atranorin
Caloplaca sp.	Soredia and isidia	-	-
Carbacanthographis marcescens (Fée) Staiger & Kalb	-	-	-
Cladonia furcata (Hudson) Schrader	Soredia	GE	Lecanoric acid fumarprotocetraric acid, atranorin, divariatic acid
Cladonia fimbriata (L.) Fr.	Soredia	GAW	Atranorin
Cladonia mauritiana Ahti & J.C David	Soredia	-	Sekikaic acid
Cladonia squamosa Hoffm.	Soredia	-	Salazinic acid, gyrophoric acid, usnic acid, atranorin
Coccocarpia palmicola (Spreng.) L. Arvidss. & Gall.	Lobus	-	No lichen substances
Collema nigrescens (Hudson) DC.	Soredia	-	Secondary metabolites: none detected
*Collema javanicum (*Müll.Arg.) Zahlbr	Soredia	-	Secondary metabolites: none detected
Collema pulcellum Ach.	Soredia	-	Secondary metabolites: none detected
Coccocarpia sp.	Lobus	-	No lichen substances
Cryptothecia striata Thor	Isidia	-	No lichen substances
Cryptothecia sp.	Isidia	-	No lichen substances
Chrysothrix sp.	-	-	-
Dibaeis sp.	Soredia	-	-
Dictyonema sericeum (Swartz) Berk.	Soredia	GE	Usnic acid
Diorygma junghuhnii (Mont. V. d Busch) Kalb, Staiger & Elix	Lirellae	-	-
Dirinaria applanata (Fee) D. D. Awasthi	Isidia and soredia	-	Atranorin, divaricatic acid
Dirinaria sp.	Isidia and soredia	-	-
Dyplolabia sp.	-	-	-
Flavoparmelia sp.	Soredia	-	-
Flavopunctelia sp.	Soredia	-	-
Fissurina elaiocarpa (A. W. Archer)	Picnidia	-	-
Flavopunctlia soredica (Nyl.) Hale	Soredia	-	-
Graphis sp. 1	Lirel	-	No lichen substances
Graphis elongate Vain.	-	-	-
Graphis immersella Müll.Arg.	Lirel	-	Stictic acid
Graphis kamojangense Jatnika & Noer	Lirel	-	-
Graphis librata C. Knight	Lirel	-	Norstictic acid
Graphis longula Kremp.	Lirel	-	-
Graphis rhizocola (Fée) Lücking & Chaves	Lirel	-	-
Graphis rustica Kremp.	Lirel	-	-
Graphis sp. 2	Lirel	-	-
Graphis sp. 3	Lirel	-	-
Haematomma persoonii (Fee) A. Massal	Apothecia	GE	Gyrophoric acid
Haematomma accolens (Stirton) Hillm.	Apothecia	GE	Barbatic acid
Heterodermia japonica (Sato) Swinscow & Krog	Soredia	GE, GAW	Atranorin, Norsticti acid chloroatranorin; zeorin, salazinic acid
Heterodermia leucomela (L.) Poelt	Soredia	-	Salazinic acid
Heterodermia rugulosa (Kurck.) Wetmore	Soredia	-	-
Hypogymnia sp. 1	Apothecia	-	Atranorin, chloroatranorin; physodic acid, physodalic acid, protocetraric acid
Hypogymnia sp. 2	-	-	Atranorin, physodic acid
Hypotrachyna sp.	-	-	-
Lecanora argentata (Ach.) Malme	-	-	-
Lecanora helva Stizenb	-	-	Atranorin dan asam chloroatranorin
Lecanora leprosa Fee	-	-	-
Lecanora sp.1	Apothecia	-	Atranorin
Lecanora sp.2		-	-
Lepraria lobificans (Nyl.)		-	-
Leptogium cyanescens (Rabenh)	Lobus and soredia	-	No lichen substances

Letharia vulpine (L.) Hue		-	-
Lobaria pulmonaria L. (Hoffm.)	Lobus and apothecia	-	Stictic Acid, desmethyl stictic acid, gyrophoric acid, tenuiorin, constictic acid, norstictic acid, peristictic acid, and methylnorstictic acid
Lobaria oregano (Tuck.) Müll.Arg.	Lobus and soredia	-	Methylstictic acid, crypto-stictic acid, cryptostic-tinolide.
Lobaria subinterversans (Gyeln.)	Lobus and soredia	-	-
Lobaria scrobiculata (Scop.) P. Gaertn.	Lobus	-	-
Maronina sp.	-	-	-
Malcomiella sp.	Apothecia	GE	Barbatic acid
Megalospora sp.1	Apothecia	GAW	Zeorin acid
Mycomicrothelia sp.	-	-	-
Megalospora sp.	Soredia	-	-
Menegazzia sp.	Soredia	-	-
Micarea prasina Fr.	-	-	-
Niebla sp. 1	Pseudothecia	GAW	Barbatic acid, Stictic acid
Nephroma sp.	Isidia and soredia	-	-
Ocelullaria sp.	-	-	-
Parmelia sp.1	Soredia and isidia	-	Atranorin, Lecanoric acid
Parmelia sp. 2	Soredia and isidia	-	Atranorin
Parmelia sp. 3	Soredia and isidia	-	Atranorin
Parmeliela sp. 1	Apothesia	-	-
Parmelia sp.1	Soredia and isidia	-	Atranorin, Lecanoric acid
Parmelia sp. 2	Soredia and isidia	-	Atranorin
Parmelia sp. 3	Soredia and isidia	-	Atranorin
Parmeliela sp. 2	Apothesia	-	-
Parmeliopsis sp.	Soredia and isidia	-	-
Parmotrema cristiferum (Taylor) Hale	Soredia		Atranorin and chloroatranorin; salazinic acid and consalazinic acids
Parmotrema dilatatum (Vain.) Hale	soredia	-	-
Parmotrema mesotropum (Müll.Arg.) Hale	Cilia and soredia	-	-
Parmotrema reticulatum (Taylor) M. Choisy	Soredia		Atranorin, chloroatranorin, salazinic acid and consalazinic acids
Parmotrema tinctorum (Delise ex Nyl) Hale	-	-	Lecanoric acid, atranorin
Parmotrema sp. 1	Cilia and soredia	-	Salazinic acid, Atranorin, Lecanoric acid
Pannaria sp. 1	-	-	-
Pannaria sp. 2	-	-	-
Peltigera sp. 1	Lobus and soredia	-	-
Peltigera polydactylon (Necker) Hoffm	Lobus and soredia	-	Tenuiorin, dolichorrhizin, zeo-rin, methyl gyrophate, gyrophoric acid,
Peltigera praetextata (Florke ex Sommerf.) Zopf	Lobus and soredia	-	-
Pertusaria sp. 1	Perithesia	-	Stictic acid
Pertusaria cicatricose Müll.Arg.	Perithesia	-	Stictic acid, constictic acid
Pertusaria texana Müll.Arg.	Perithesia	-	-
Pertusaria sp. 2	Perithesia	-	-
Pertusaria sp. 3	Perithesia	-	-
Phaeographis adinoconspicua (Fée) Müll.Arg.	Lirel	-	-
Phaeographis dendritica (Ach.) Müll.Arg.	Lirel	-	-
Phaeographis dendroides (Leight.) Müll.Arg.	Lirel	-	-
Phaeographis intricans Nyl.	Lirel	-	-
Phaeographis lobata (Eschw.) Müll.Arg.	Lirel	-	-
*Phaeographis schizoloma (*Müll.Arg.)	Lirel	-	-
Paeographis sp.	Lirel	GE	Barbatic
Physcia sp.	Soredia and isidia	GAW	Lecanoric acid
Pseudocyphellaria aurata (Ach.) Vain.	Lobus	GAW	Gyrophoric acid
Pseudocyphellaria crocata (L.) Vain.	Lobus	GAW	Barbatic acid
Pseudocyphellaria sp.	Lobus	-	-
Psora pseudorusellii	Soredia and isidia	-	-
Pyrenula subdunata	-	-	-
Pyrenula sp.1	Perithesia	GAAn, GAW	Barbatic acid, usnic acid, salacinic acid, gyroporic acid
Pyxine sp. 1	Apothesia	GAAn, GAW	Barbatic acid, usnic acid
Pyxine sp. 2	-	-	-
Ramalina celastri (Spreng.) Krog & Swinscow	Soredia	GE, GAW	Iso-usnic acid and usnic acid
Ramalina farinacea (L.) Ach.	Soredia	GE	Usnic acid
Ramalina pollinaria (Westr.) Ach.	Soredia	GAAn	Usnic acid

Ramalina sp.	Soredia	GAW	Iso-usnic acid and usnic acid
Relicina mallesiana(Hale) Hale	Soredia	-	-
Relicina sp. 1	Soredia	-	-
Relicina sp. 2	Soredia	-	-
Rhizocarpon sp.	Perithesia	GE	Rhizocarpic acid
Sarcographa cinchonarum Fee	Hysterothesia	GE	Stictic acid
Sarcographa tricosa (Ach.) Mull. Arg.	Hysterothesia	GAAn	Salazinic acid, stictic acid, evernic acid
Stereocoulon sp.	Apothecia		Decanoic acid
Sticta dissimulate	Picnidia and soredia	-	Gyrophoric acid
Sticta lingulata Vain.	Picnidia and soredia	-	Gyrophoric acid
Teloschistes flavicans (Sw.) Norman	Pseudothesia	-	Parietin, teloschistin, parietinic acid caloploicin
Usnea articulate (L.) Hoffm	Isidia and pseudothesia	GAW GAoT	Barbatik, physodalic, lecanoric, physodalic Chloroatranorin
Usnea baileyi (Stirton) Zahlbr.	Isidia and pseudothecia□	GE GAoT	Usnic acid Grayanic, diffractaic
Usnea ceratina Ach.	Isidia and pseudothecia□	GAW GAoT GE	Usnic acid Divaricatic Gyrophoric, lobaric, isousnic
Usnea dasypoga (Ach.) Shirley	Isidia and pseudothecia□	GAW	Usnic acid, diffractaic acid
Usnea subfloridana Stirton	Isidia and pseudothecia□	GE GAW	Usnic acid Gyrophoric
U. flexilis Stirt	Isidia and pseudothecia□	GAW GE GAAn	Stenosporic, isousnic Retigeric, diploicin, and usnic acid, Leucotylin
Usnea hirta (L.) Weber ex F.H Wigg.	Isidia and pseudothecia□	GE	Usnic acid
Usnea trichodea Ach.	Isidia and pseudothecia□	GAW	Usnic acid

A B

Figure 2. Rare lichens. A. *Dictyonema sericeum* (Sw.) Berk, B. *Usnea longisima* Ach

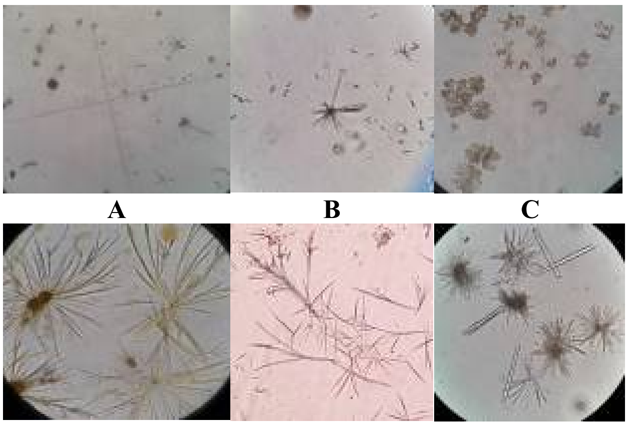

Figure 3. Several displays of lichen's acid crystals: A. Atranorin acid, B. Barbatic acid, C. Baeomycesis Acid (G.A.An), D. Gyrophoric acid (G.E), E. Lecanoric acid (GE), F. Usnic acid (G.E)

In general lichens in Kamojang geothermal area contains atranorin, usnic acid**,** gyrophoric and barbatic (Tabel 1). Those lichenic acid has reported are have potential for medicinal properties (Ashina and Shibata 1954; Cobanoglu et al. 2010; Crawford 2015). Such as usnic acid and salizilic acid were used for antiinflammation and analgesic action, and usnic acid has used as antifungal and antibiotic (Rankovic et al. 2008; 2007b). Beside that isodivaricatic, 5-propylresorcinol acid, divaricatinic acid and usnic acid which are contained in *Usnea florida* var. *rigida*. Acharius have proven good for antimicrobial activity against fungi *Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* growth (Fazio et al. 2007; Kekuda et al. 2016). Usnic acid showed also significant antibacterial activity against *Bacillus cereus, B. megaterium, Staphylococcus aureus* and *Klebsiella pneumoniae* (Saenz et al. 2006; Kukeda et al. 2016).

Atranorin isolated from lichen demonstrated an approximate and relatively strong antimicrobial activity against bacteria *Bacillus mycoides, B. subtilis, Staphylococcus aureus, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae* and fungi *Aspergillus flavus, A. fumigatus, Botrytis cinerea, Candida albicans, Fusarium oxysporum, Mucor mucedo, Paecilomyces variotii, Penicillium purpurescens, P. verrucosum, Trichoderma harzianum* (Ranković et al. 2007a, 2007b; 2008; Kukeda et al. 2016). It is reported that *Acarospora, contain* norstictic acid. rhizocarpic acid gyrophoric acids epanorin acids. These acid have reported inhibit from the growing *of B. subtilis* and *S. aureus (*Řezanka and Guschina 1999)

So, from 133 species found in Kamojang Geothermal Area, there are, twenty-three (23) genus have been identified as medicine lichens. The genus has possible good potentially for medicine base on literature study were *Acarospora, Bulbothrix, Cladonia, Collema, Coccocarpia, Flavoparmelia, Graphis, Heterodermia, Hypogymnia, Leptogium, Lobaria, Lecanora, Lasallia, Nephroma, Parmotrema***,** *Pseudocyphellaria, Peltigera, Parmelia, Pertusaria, Physcia, Ramalina, Sticta,* and *Usnea*

Lichens have been used in traditional medicine since the time of the first Chinese and Egyptian civilizations (Chopra 1958; Noer 2013). Their utilization in folklore as medicine has been cited in different pharmacopeias of the world (cf. Nisyapuri et al. 2018). During the middle-ages lichens figured prominently among the herbs used by medicinal practitioners (Hale 1983). The use of lichens in medicine can be traced back to antiquity. *Evernia furfuracea* has been found in an Egyptian vase belongs to 18^{th} Dynasty (1700-1600 BC) was used as a drug (Crawford 2015; Lal 1990). The literature review and records of medicinal plant lore of Indonesia show the word ‘janggot kai, rusuk angin and kayu angin' are used for lichen *Usnea* for long time ago, a text where the first authentic record of ‘jamu’ (medicine) has been described (Noer 2013). The Java names of “rusuk angin” were later identified to several species of Parmelioid lichens, such as

A B C D

E F

Figure 4. The process of coloring clothing raw materials with lichens. A. *Parmotrema tinctorum,* B. *P. tinctorum* in ammonia solution after two weeks, C. Wool, D. Brown color produced by *Usnea* reddish up to dark purple produced by *P. tinctorum,* E. Cotton was dyed with *P. tinctorum* after a couple of months in ammonia solution, F. The lichen dyes process and dyed wool result

Usnea longisima. U. barnata, U. missamensis. U. dasypoga and *Telosichtes.* The vernacular name jamu widely used in Indonesian traditional medicine, an ancient system of locally Indonesia medicine, for different disease and disorders, for example, headache, skin diseases, urinary trouble, boils, vomiting, diarrhea, dysentery, heart trouble, cough, fever, leprosy and as a blood purifier.

Lichen dyes

Dye colors produced from *Parmotrema tinctorum* using ammoniac extracts were brownish red, dark red and purple. The color change of *Parmotrema tinctorum* ammoniac extracts occurred after 1st until 3rd weeks of fermentation. *P. tinctorum* had brownish red in 1st week and dark red after 3rd week of fermentation. Purple colors occurred in 4th week and dark violet color had been stable since 5th week of fermentation. While *Usnea* spp.**,** produced brown color dyes and various brown since 5 days of fermentation up to 4th week. Base on the literature study, lichens at Kamojang geothermal area which has potential for dyes are *Parmotrema, Usnea, Lobaria, Peltigera,* and *Hypogymnia.*

Ammonia fermentation methods (AFM) is the best method to get a wide range of colors such as pink, violet, orange, grey, brown and yellow. Livelihoods natural dyes from lichens *Parmotrema* very interesting and will be important in the use of dyes that are environmentally friendly in industry that would reduce the pollution of water/river by dyes from industry.

In Ammonia extraction, lichens that have a C+ response are best for purple and violet dyes (Allen 2014). Spot test has done to *Parmotrema s*pp. giving respond C+ red. *Parmotrema tinctorum* ammoniac extracts produced greyish violet. This was in accordance with studies by Casselman and Terada (2012) who stated that *P. tinctorum* produced purple color on fabric and thread through ammoniac fermentation. Such different colors shown from lichen ammoniac extracts were related to compounds in the lichen. Produced colors indicated the presence of particular compounds in *Parmotrema tinctorum* and *Usnea baileyi* species that reacted with ammoniac and aquades*t.*

Purple color was produced presumably due to lecanoric acid in the *Parmotrema tinctorum* species. Lecanoric acid is p-depside that were hydrolyzed to orselic acid and undergoes a series of chemical reaction to form ‘orcein’ color precursor (Shukla et al. 2014). Salgado et al. (2018) reported that *Parmotrema* contains gyrophoric acid. It has been used as a purple and red dye for thousands of years, mainly in the northern hemisphere

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