DIVERSITY, DISTRIBUTION AND CHEMOTAXONOMY OF MACRO LICHENS AT MESILAU NATURE PARK, SABAH

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ABSTRACT

Mesilau Nature Park is a hotspot for macro lichens but no detail research is done on the distribution and diversity of this organism. Since 1993, there are no further records or research on lichens at Mesilau Nature Park. Hence, this project is started with the objective to survey for macro lichen's distribution and diversity, followed by production of a checklist of macro lichens at Mesilau Nature Park and to compare similar occurring genera at three different sites, Mesilau Nature Park, Mount Alab and Mount Trus Madi. Mesilau Nature Park was divided into four different sampling zones. As Zone 1 is a small patch, it is excluded from the distribution and diversity measures but a small checklist was produced as an outcome. Meanwhile, the sampling area and distance was standardized for the zones 1, 2 and 3. This is done to measure their distribution and diversity of macro lichens. At the end of the sampling, in total 143 samples of macro lichens was collected with 21 genera recorded. Three different diversity index, Shannon-wiener diversity index, Simpson's Diversity index and Kruskal-Wallis test, were calculated based on each sampling zones. Zone 3 was recorded to have the highest diversity with total of 16 genera (35 samples) recorded. Meanwhile, Zone 4 records the lowest diversity with only 5 genera (7 samples) recorded. The extant of similar occurring genera of macro lichens at three different sampling sites, Mesilau Nature Park, Mount Alab and Mount Trus Madi, made it possible for comparison between these three sites to be carried out. Three species was choosed for comparison purpose, where two species from the genus *Cladonia* and one species from the genus *Stereocaulon*. Chemical profiling using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography were performed on all nine samples. Outcome of the profiling indicates certain amount of similarly occurring compounds within samples of same species. Abundance of compounds within lichen material may be influenced by different environmental parameters and climatic factor at each sampling sites. Therefore, research on distribution of macro lichens and accumulation of lichen compounds within lichen material will be very beneficial in further understanding macro lichens.

ABSTRAK

KEPELBAGAIAN, DISTRIBUSI DAN TAKSONOMI KIMIA LIKEN DI TAMAN SEMULAJADI MESILAU, SABAH

Mesilau Nature Park merupakan hotspot untuk liken, tetapi tidak banyak kajian atas kepelbagaian organisma ini di kawasan tersebut. Sejak tahun 1993, tiada rekod dan kajian dilakukan ke atas liken. Oleh sebab itu, kajian ini dimulakan dengan objektif untuk mengkaji distribusi dan kepelbagaian liken, menghasilkan checklist dan melakukan perbandingan di antara tiga kawasan iaitu Taman Semulajadi Mesilau, Gunung Alab dan Gunung Trus Madi. Taman Semulajadi Mesilau seterusnya dibahagikan kepada empat zon persampelan. Kesemua empat zon persampelan merekod distribusi liken yang tinggi. Oleh sebab, Zon 1 merupakan satu kawasan yang kecil, zon ini dikeluarkan dari perbandingan dari segi distribusi dan kepelbagaian. Manakala, zon 2, zon 3 dan zon 4 telah diselaraskan saiz tempat dan jarak kajian untuk tujuan perbandingan tanpa berat sebelah. Secara keseluruhan 143 sampel telah dikumpul dengan 21 genera direkod. Berdasarkan keputusan persampelan, tiga indeks, Shannon-wiener diversity index, Simpson's Diversity index and Kruskal-Wallis test, telah dikira untuk memahami kepelbagaian liken sepenuhnya. Secara amnya, Zon 3 mencatat indeks tertinggi dengan jumlah genera sebanyak 16 (35 sampel). Zon 4 telah mencatat indeks terendah dengan jumlah genera sebanyak 5 (7 sampel). Kewujudan liken yang serupa dari segi genus dan spesies, di tiga tempat berlainan, mendorong kepada perbandingan. Tiga spesis telah dipilih, dua spesis dari genus *Cladonia* dan satu spesis dari genus *Stereocaulon*. Profil kimia menggunakan Thin Layer Chromatography (TLC) dan High Performance Liquid Chromatography (HPLC) telah dilaksanakan ke atas semua sembilan sampel. Keputusan melalui profil menunjukkan kehadiran sebatian yang sama untuk spesies liken yang sama. Ketinggian puncak menunjukkan kuantiti sebatian di dalam sesuatu liken dan difahami, ketinggian setiap kompoun dan kuantiti sebatian dipengaruhi oleh parameter persekitaran dan factor iklim di setiap kawasa persampelan. Oleh sebab itu, penyelidikan berterusan mengenai distribusi dan kepelbagaian liken perlu diteruskan untuk memahami organisma ini dengan lebih mendalam.

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LIST OF SYMBOLS AND ABBREVIATIONS

^oC	degree Celcius
%	percent
M	metre
nm	nanometre
mm	millimetre
cm	centimetre
ha	hectare
µl	micro litre
UV	ultraviolet
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
MeOH	methanol
EtOAc	ethyl acetate
Hex	Hexane

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Growth of macrolichen depends greatly on the surrounding's climate and micro-climate. Necessities such as right humidity, temperature, light intensity and presence of air pollutant are needed by macrolichens to grow healthily. These necessities are not fulfilled at many places, which contribute to the absence of many macrolichen species. But Borneo Island, especially the state Sabah, has high number of highlands and mountains which is very suitable for the growth of macrolichen. Recent macrolichen research proven to be very profiting not only for the importance of the ecosystem but for humans as well (Antoine, 2004).

Sabah has their own tropical highland such as the Crocker Range which has many peaks over 1500 m, including Mount Kinabalu with the height of 4095 m and Mount Alab with the height of 1964 m meanwhile Trus Madi Range consist of Malaysia's second highest mountain, Mount Trus Madi with the height of 2642 m (Peter *et al.*, 2002). Each of these mountain regions has montane forest. Montane forest usually grows 1000 m and above from the sea level. Humidity, temperature, light intensity and the clean air provide a great home for macrolichens.

Identifying and differentiating the macrolichens from each other are very important. Most of the macrolichens can be distinguished based on their morphological characteristics but some macrolichens species resembles other species of macrolichen quite closely. This is where new method is needed for taxonomically characterizing each macrolichen species. By doing chemical profiling of the macrolichens, compounds that were obtained can be used to classify them more correctly and systematically. This research is important because the result of this research were very helpful for future macrolichenologist and macrolichen chemist in identifying macrolichens of Mesilau Nature Park.

1.2 Justification of Study

The outcomes from this research will be very helpful for future macrolichenologist that wants to conduct research at Mesilau Park. Sipman (1993) had recorded up to 286 species of macrolichenized fungi on Mount Kinabalu and along the Mesilau Trail. Even though it is a near complete list on the macrolichen flora at Mesilau Park, there is no record on their locations. In this research, the distribution and diversity of macrolichens at Mesilau Nature Park and Mesilau Trail was accessed. From the samples of macrolichen flora collected, Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were carried out upon chosen macrolichen group to determine their correct systematic and taxonomy.

1.3 Objectives

The objectives of this study were as following:

1. To determine the distribution and diversity of macrolichens at Mesilau Nature Park.

2. To prepare a check list of macrolichen at this location.

3. To determine chemical profiles of similarly occurring macrolichen among Mesilau Nature Park, Mount Alab and Mount Trus Madi.

CHAPTER 2

LITERATURE REVIEW

2.1 Mutualistic Relationship

For a non-expert's eye, macrolichen only is seen as plant-like organism or fungal species. It certainly suits the name given to this organism. 'Lichen' means 'a kind of plant' in Latin. To describe an outgrowth from a bark of olive trees, the word 'lichen' (lie 'ken) was introduced by Theophrastus in the Greek literature in about 300 BC (Hawksworth, 1984). Until the end of the 16^{th} century, macrolichens are only identified based on their morphological characteristics and often wrongly grouped together with mosses, fungi or seaweeds. Advent of microscopes in the beginning of 18^{th} century, enabled researchers to look closer into macrolichen's anatomical structures. This lead to the revelation of their dual character, consisting algal and fungal partners (Lindsay, 1856).

Macrolichens are a great example of composite organisms. Macrolichen is formed when there is a mutualistic relationship between fungus, mycobiont, with a photosynthesis partner, photobiont, which are usually either a green algae or fungus or cyanobacteria. The algal or cyanobacteria partner possess the green pigment chlorophyll, which enables macrolichen to harvest the sunlight's energy, carbon dioxide and water, to produce carbohydrate through photosynthesis. Most fungi or algae could not produce amino acids by directly absorbing from nitrogen gas in the atmosphere, but some cyanobacteria can. In return, the fungal partner, mycobiont, provides a body or home for the photobiont to take shade under them, so that they would not dry out under strong sunlight. Survival of this organism is largely dependable on the photosynthetic product of the photobiont. The term symbiosis is the best term to describe the association shared by the mycobiont and photobiont in the macrolichen. In total, there are almost twenty thousand macrolichens are found and noted by researchers till today. Each of these macrolichens involves different fungal partners, but most macrolichens have same algal partner. This shows that not many species of algae involve in the symbiotic relationship between funguses to form macrolichen. Even though there is involvement of many different kinds of fungal partners, 98 % of the fungal partner consists of Ascomycota and the remaining 2 % are Zygomycota (Hawksworth and Hill., 1984).

Macrolichen's body or thallus can be classified into general three different types. Macrolichen thallus that looks like a small miniature shrub-like structure is classified as fruticose type. Foliose type macrolichen produce leaf-like structures flattened and have layers of upper and lower surface of lobed thalli. Meanwhile, crustose type macrolichens form crust over may be wedged in between the crystals of rocks even can be buried among the tree bark (Hawksworth *et al.,* 1995).

Reproductions of macrolichens are very complicated and not many researches are done on this subject. The process of producing new macrolichen of the same species involves the mutualistic relationship of the same photobiont and mycobiont. Amazingly, macrolichen species had undergone quite an evolutionary change that enables macrolichen to reproduce sexually and asexually. Reproducing sexually involve the formation of fruiting bodies in macrolichen. Macrolichen with Ascomycota fungal partner able to produce disc shaped fruiting bodies, which also known as apothecia. The spores produced by the macrolichen are the spores of the fungal partner only. The role of the algal partner in the reproduction of macrolichen is only to provide energy for reproduction. In the end, algal partner would not have any energy left for its survival or reproduction. Spores produce by the macrolichen can be dispersed by wind, rain or with the assistance from animals. Upon germination, the macrolichen spores would have two methods of survival. Onemethod is the spore will share the algal partner of other macrolichen growing there or they will find suitable algae. Not able to do so, the spore could not survive long. Meanwhile, asexual reproduction of macrolichen is where brittle macrolichen parts are broken and dispersed by wind, rain or by insects and birds. The fragment of the macrolichen that broken off contain mycobiont together with the photobiont. Hence, the survivability of the fragments is considerably very high (Longton, 1988).

Researchers have not been able to establish with any certainty when and how fungus-alga associations evolved, although macrolichens must have evolved more recently than their components and probably arose independently from different group's photobionts which compose of fungi and algae or fungi and cyanobacteria. Mycobiont in the macrolichen determines the basic appearance of the macrolichen thallus, fruticose, foliose, squamulose or crustose (Nash, 2001). Structure of macrolichen can be divided into four main parts, the cortex, algal layer, medulla and basal attachment. The outer layer of macrolichen thallus is known as the cortex. Cortex layer is mostly covered with the fungal partner, where the cells are thicker arranged more closely compared to other part of the macrolichen. This outer layer also serves as the macrolichen's defense which protects the mycobiont and photobiont from external cause damages. Meanwhile the algal layer can consist of either the green algae or cyanobacteria. Colour of macrolichen can indicate the type of algal partner. If macrolichen contains green alga, it was in a bright green colour. But if the macrolichen contains cyanobacteria, it can give out dark green, brown or black colour. This colour indicator only applies for the macrolichens that have algae layer. In some macrolichens, layers between photobiont and the mycobiont could not be differentiated. These types of macrolichen are known as the jelly macrolichen. This is due to the growth form that is gelatinous with one big layer mixed with photobiont and mycobiont (Nash, 2001). Loosely woven layer of fungal filaments are known as the medulla. Medulla forms the layer after algal layer. Medulla results in the formation of soft, spongy and cotton-like form underneath the cortex. Lastly, basal attachment is the lowest part of macrolichen. As different form and type of macrolichen, can attach to different substrate types, every macrolichen will have their own special anchor. Macrolichen's anchor can be divided into two, holdfast and rhizines. Macrolichen's with holdfast are generally grows on rock. Holdfast structures grow out of the macrolichen thallus, and attach it to the desired substrate. Meanwhile rhizines are fungal filaments that are responsible to attach the macrolichen to its substrate. Rhizine does not have the ability to transfer water and minerals as the roots of vascular plant. Rhizine's main role would be to hold the macrolichen down to the substrate it is sitting on (Longton, 1988).

2.2 Diversity of Macro Lichen

It has been estimated that 20% of the more than 64,000 known fungal species are macrolichenised fungi. Out of it, nearly 20, 000 macrolichenous species known by humans at present dominate the approximately at 8% of earth's ecosystem (Hawksworth *et al.*, 1995). The specific range of distribution of macrolichen species is not known. Macrolichens can be found from the Equator to the Polar Regions and even on a higher altitude. Most marine species colonise on rocks immersed in streams and lakes but none are found living in sea water (Paolo, 1998). For their ability to grow anywhere on earth surface, even in extreme environments, they are called pioneers (Piervittori, 1998). Galloway (1992) has estimated about 13, 500 species of macrolichen are currently accepted and approximately 20, 000 species would be the actual world total. Thus, compared to other fungal groups, macrolichenized fungi are much better described and identified. Out of the large number of discovered macrolichenized fungi, up to 60-80%, which make up 5% of overall fungi count, are fully described (Heywood, 1995).

Growth of macrolichen could not be limited with environment or weather. Most of the existent macrolichens have the ability to grow in an environment most plants could not survive. Giving to their ability to flourish in inhabitable environments, they become the pioneer species of a habitat. The growth of macrolichen in a particular habitat, such as bare rocks or severely burnt areas, will make the soil to be more suitable for the growth of other plant species (Awasthi, 2010). As time goes by, the growth of macrolichen were limited as growth of other plant species bloom. Growth substrate of a macrolichens have unlimited possibilities, as macrolichens are found growing on cooled and solidified larval flows, frozen soils at the polar regions and even on the marbles of historical ancient ruins. Even though, macrolichens in the tropical and subtropical rain forest grow abundantly due to the presence of high humidity, macrolichens can adapt to survive under great circumstances such as heat, cold and even drought (Awasthi, 2010). Nevertheless, growths of macrolichen are limited or even some cases none, in the industrial areas. This is because macrolichens are sensitive to the changes in atmospheric concentration of oxides of sulphur and other pollutants.

Though the macrolichens are found in abundant, they have not evolved in great variety of families and genera. Climate and substratum is the limitation of growth and distribution of macrolichens. Although nearly all the macrolichen families are well represented, recent studies on the distribution of macrolichen shows that, certain families are more abundant in some domain. In the tropical forests, the occurrence of macrolichens can reach up to one-third and one-half of the world macrolichen diversity (Aptroot and Sipman, 1997).

One-fifth of the earth's surfaces are occupied by the tropical forest. Since the tertiary period, tropical forest had been the dominant vegetation over large areas of the landmass. Climate of tropical rain forest are very wet, this is because the annual precipitation of the forests can reach up to 250 cm. With rain and sun all day, throughout the year, the relative humidity is always high. The temperatures during the day are between 30°C - 35 °C and during the night, the temperature can drop to between 20°C - 25 °C. Even though, tropical rain forest did not have seasonal period, they do have months where they can be warmer or colder compared to other months. Equatorial climate is the name given to the rainforest of this climate as tropical climate are usually and only can be found along the equator. Due to this climate, macrolichens are known to grow in abundance with wide distribution and large diversity. With this amount of humidity, temperature and sun light, macrolichens does not face any difficulties in growth, development or reproduction.

Borneo Island is the third-largest island in the world. Being one of the world's richest biodiversity filled island, Borneon Island is known to be the treasure chest for world's macrolichenologist or lichen chemist. With 75, 370 hectares (ha), Kinabalu Park have the astonishing variety of flora and fauna. With mean annual rainfall reaching 2380 mm, Kinabalu Park is the heaven for lichenologists. Via the means of field survey, and referring to herbarium materials and literature records, 286 species of macrolichens are recorded on Mount Kinabalu (Sipman, 1993).

2.3 Importance of Macro Lichen

Macrolichen make up a large part of the world's ecosystem and their importance in the ecosystem could not be omitted. Due to their general and tough characteristic, macrolichen could grow in any climate and environment. They are often the pioneer species in invading a hostile environment. Macrolichen not only is able to grow in a sterile environment, they also able to survive in an environment with extremely minimal amount of water. When the macrolichen dies and decays, macrolichen cell and tissue nourishes the soil which serves as a nourishment for the growth of other plant species. Other than playing a major role in the succession process, macrolichens are also important in nitrogen cycle and in the soil formation.

Nitrogen is very important to the entire living organism. Nitrogen plays an important role to the biotic and abiotic factors of the earth's system. Nitrogen is the macro nutrient which the organism obtains from the atmosphere. Nitrogen make up a large part of all living cells, protein, enzymes and nitrogen also plays important role in metabolic processes. In plants, nitrogen is needed as a regulatory of photosynthesis process. Chlorophyll, the green pigment of the plant that is responsible for photosynthesis in plants, made up of nitrogen. Nitrogen helps the plant to grow rapidly, increase fruit and seed production and in agricultural sectors, nitrogen increases plant health ad increase crop's yield. Meanwhile, animals need nitrogen as nitrogen make up their cellular membrane. Nitrogen plays a big part of nucleic acid, amino acids and proteins. Many macrolichens are known nitrogen fixers that are available abundantly in the ecosystem. The known macrolichens are able to extract nitrogen from the atmosphere, utilize it and pass it on to other organisms. Cyanobacteria in the macrolichens are the one responsible in fixation of nitrogen. Cyanobacteria able to produce ammonium from N_2. *Calothrix, Stigonema, Nostoc* and *Scytonema* are some of the many taxa of cyanobacteria that are known to fix nitrogen. Cyanobacteria in the macrolichen will get hold of the free nitrogen in the NO_2 and convert them into nitrate (Brown and Dalton, 2002). Converted nitrates were absorbed by the macrolichen itself to build proteins. Animals obtain nitrogen from macrolichen by eating them. Primary consumers, herbivores, consume macrolichen and break down the protein to obtain the needed nitrogen. Dead macrolichens, will release nitrites or nitrates to the soil which were utilize by plants (Antoine, 2004).

The important role of macrolichens as the pioneer species to the new and sterile environment is to form soil. This significant contribution of macrolichen in the ecosystem is the starting mechanism for the colonization of other plant species. The macrolichens that are capable of making this possible are known as the rock macrolichen, as these macrolichens grow on rocks. Since macrolichen is one of the first organisms to grow on bare rocks, they play important role in soil formation by slow degrading the rock substrate. Pedogenesis or the soil formation is known to happen in two different ways. One of the ways is by the means of chemical substance. Macrolichen secrets chemical substances which initiate the degradation of a rock. Chemicals such as carbonic acid, depsides and depsidones are known to cause fast weathering process on rocks especially rocks that contain calcium carbonate (Syers and Iskandar, 1973). Another way of degradation of rock would be through the mechanical action. Degradation of mechanical action starts through the penetration of the macrolichen's hyphae. Breaking of the rock substrate happens through the expansion and contraction of the macrolichen's hyphae or crustose thalli. The expansion and contraction is caused by the gain and loss of water (Fry, 1927). The breaking down of the rock substrate forms the soil, which become the medium for the growth of other plants. In the primary succession, the soil and humus of the dead macrolichen parts, creates a better and fertile area for plant growth. Macrolichens can also lead to soil formations at volcanic rocks at Hawaii and even at the polar region. Ground-dwelling macrolichens are found to be important in stabilizing bare ground and they prevent erosions.

Caribou and reindeer are well known animals that eat macrolichen as one of their food source during winter. This phenomenon is well documented. In the winter, caribou and reindeer are well-known to dig craters in the snow in the effort to find macrolichen. This macrolichen, also known as Caribou moss grows in the northern regions around the world. Due to its tough exterior, the macrolichen's tissue isn't that easily damaged by the cold weather. During the winter, absence of water causes the macrolichen to dry out and enter the dormant stage. This caused by the absence of water and light. These macrolichens still able to grow back even after a long period of dormancy. Survivability and presence of macrolichen during the winter, is the reason why the 90% reindeer and caribou's winter diet made up of this macrolichen. Even though the macrolichen tissues contain low concentration of protein, but they are high in carbohydrate. Caribou ingest these macrolichens to get energy to produce more body heat. Apart of being food source for animals, macrolichens are also known to provide water during the winter (Thomas and Rosentreter, 1992). Macrolichens from the genus *Bryoria* are good absorber of solar radiation. This is because they have dark-coloured exterior. These macrolichens are tending to grow in the canopy, where they provide liquid to the shrews, flying squirrels and birds. Macrolichens are also known to be used by animal as basic nest building material. Birds and small mammals choose macrolichen because of their insulating, flexible and soft properties. Hummingbirds are the common ones. Many

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