



UNIVERSITI PUTRA MALAYSIA

**DETERMINATION OF AIR QUALITY USING INDEX OF ATMOSPHERIC
PURITY AND ANTIOXIDANT ACTIVITY IN LICHENS FROM GUNUNG
JERAI AND SUNGAI PETANI, KEDAH, MALAYSIA**

NUR SYUHADA BINTI ROSLI

FS 2021 6



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PURITY AND ANTIOXIDANT ACTIVITY IN LICHENS FROM GUNUNG
JERAI AND SUNGAI PETANI, KEDAH, MALAYSIA**

By

NUR SYUHADA BINTI ROSLI

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

November 2019

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DEDICATION

This thesis is dedicated to

The lichenologist and conservationist, who truly work to protect and preserve the biodiversity of the Land.



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

DETERMINATION OF AIR QUALITY USING INDEX OF ATMOSPHERIC PURITY AND ANTIOXIDANT ACTIVITY IN LICHENS FROM GUNUNG JERAI AND SUNGAI PETANI, KEDAH, MALAYSIA

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November 2019

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Lichens are excellent bio-indicator for air quality assessment and they can be utilised as natural sources of antioxidant. Index of Atmospheric Purity (IAP) is an ecological index that uses lichen frequency to quantify environmental conditions, primarily air pollution. The IAP technique is considered new and has not been used extensively in Malaysia. Thus, the aims of the study are to: (i) determine the lichen diversity index of Gunung Jerai(GJ) and Sungai Petani (SP) using Shannon's Index (ii) determine and compare the Index of Atmospheric Purity score of forested site; Gunung Jerai and urban site; Sg. Petani (iii) determine the antioxidant activity of selected lichens species. The lichens were collected at GJ (80 m, 300 m, 600 m, 900 m &1200 m) and SP within 5 x 10 m sampling site size. Twelve trees were chosen at each sampling site and the tree barks were marked with 10 x 50 cm sampling grid. Lichens attached at tree barks above 100 cm from the ground were collected. Standard morphological and chemical tests were conducted for lichen species identification. As for antioxidant evaluation, total phenolic content and total flavonoid content were assessed with DPPH assay. The lichen diversity index of GJ (3.8862) is higher compare to SP (1.5942). The air quality of sampling area, GJ and SP were determined by IAP score, low score indicated high level of pollution. Based on the Quality Level of IAP Table, GJ is classified as low level of pollution (IAP score of 42.12) and SP (8.3) as very highly polluted. Lichens IAP levels are predominantly affected by lichen habitat disturbance and air pollution. In SP, close proximity of the sampling to the main road traffic may contribute to be cause of the low IAP reading. Meanwhile, in GJ, recreational activities may contribute to the habitat disruption of lichens and low IAP score. For antioxidant activity, the antioxidant activity of acetone extract of Ramalina farinacea is highest among other species. The antioxidant content levels are as follow: Ramalina farinacea >Cladonia sp.>Parmelia sp.>Cladia aggregata. Overall, both GJ and SP are vulnerable to air pollution and lichen species reduction due to habitat disturbance. Effective enforcement should be taken by Department of Forestry in order to preserve the pristine conditions of GJ. Besides, the lichens potential as antioxidants source should be explored extensively in terms of application and commercialization.

Keywords: Lichen, Diversity, Kedah, Antioxidant, IAP



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN KUALITI UDARA MENGGUNAKAN *INDEX OF ATMOSPHERIC PURITY* DAN AKTIVITI ANTIOKSIDAN LIKEN DARIPADA GUNUNG JERAI DAN SUNGAI PETANI, KEDAH, MALAYSIA

Oleh

NUR SYUHADA BINTI ROSLI

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Liken adalah bioindikator yang sangat baik untuk penilaian kualiti udara dan ia boleh dimanfaatkan sebagai sumber antioksidan semula jadi. *Index of Atmospheric Purity* (IAP) adalah indeks ekologi yang menggunakan frekuensi liken untuk menilai keadaan udara persekitaran, terutamanya pencemaran udara. Teknik IAP masih baharu dan tidak digunakan secara meluas di Malaysia. Justeru, kajian ini dijalankan untuk: (i) menentukan diversiti liken di Gunung Jerai (GJ) dan Sungai Petani (SP) menggunakan Indeks Kepelbagaian Shannon (ii) menentukan and membandingkan skor IAP di kawasan hutan; GJ dan kawasan Bandar; SP (iii) menentukan aktiviti antioksidan liken yang terpilih. Liken disampel daripada kaki bukit GJ ke puncak (80 m, 300 m, 600 m, 900 m & 1200 m) dan di taman bandar SP dalam saiz tapak sampel 5 x 10 m. Dua belas pokok telah dipilih di setiap kawasan sampel dan batang pokok ditandakan dengan grid sampel 10 x 50cm. Hanya liken yang terdapat di dalam grid sampel yang terletak 1 m daripada tanah diambil. Ujian morfologi dan kimia dijalankan untuk mengenalpasti spesies liken. Adapun penilaian antioksidan, jumlah kandungan fenolik dan jumlah kandungan flavonoid dinilai dengan ujian DPPH. Diversiti liken yang dinilai menggunakan Indeks Kepelbagaian Shannon di GJ (3.8862) lebih tinggi berbanding di SP (1.5942). Kualiti udara di kawasan persampelan, GJ dan SP ditentukan oleh skor IAP, skor rendah menunjukkan tahap pencemaran yang tinggi. Berdasarkan Tahap Kualiti Jadual IAP, GJ diklasifikasikan sebagai kawasan yang mempunyai tahap pencemaran yang rendah (skor IAP 42.12) sedangkan SP (8.3) mempunyai tahap pencemaran yang sangat tinggi. Tahap IAP dipengaruhi oleh gangguan habitat liken dan pencemaran udara. Di SP, kawasan persampelan yang berdekatan dengan laluan trafik dan jalan utama boleh menyumbang kepada bacaan IAP yang rendah. Sementara itu, di GJ, aktiviti rekreasi manusia yang tidak terkawal adalah faktor yang menyumbang kepada gangguan habitat liken dan skor IAP yang rendah. Aktiviti antioksidan daripada ekstrak aseton *Ramalina farinacea* dianggap paling tinggi dalam kalangan liken yang diuji. Tahap aktiviti antioksidan adalah seperti berikut: *Ramalina farinacea*.> *Cladonia* sp.> *Parmelia* sp.> *Cladia aggregata*. Secara keseluruhannya, kedua-dua kawasan, GJ dan SP terdedah kepada risiko pencemaran udara dan penurunan spesies liken kerana kerosakan habitat

semulajadi yang tidak terkawal. Penguatkuasaan yang berkesan oleh Jabatan Hutan perlu dilaksanakan untuk memelihara keaslian GJ. Selain itu, potensi liken sebagai sumber antioksidan perlu diterokai terutamanya tentang kaedah aplikasi dan pengkomersilan.

Kata Kunci: Liken, Diversiti, Kedah, Antioksidan, IAP



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With the name of Allah the Most Compassionate and Most Merciful

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To end with, I am truly delighted, for this research would benefit to others.

APPROVAL

I certify that a Thesis Examination Committee has met on 20 November 2019 to conduct the final examination of Nur Syuhada Binti Rosli on her thesis entitled “Determination of Air Quality Using Index of Atmospheric Purity and Antioxidant Activity in Lichens from Gunung Jerai and Sungai Petani, Kedah” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student is awarded the Doctor of Philosophy. Members of the Thesis Examination Committee were as follows:

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Gunung Jerai also known as 'eye of Kedah' is located in Gurun in Kuala Muda District, Kedah. The massive limestone outcrop is one of the highest peaks in Kedah which was formed during Cambrian era. Sanctuary one of the most pristine rainforests in the world, it is teeming with exotic flora and fauna including lichens. Lichens are symbiotic mutualism relationship of a fungus (mycobiont) and photosynthetic partner which are either algae or a cyanobacteria (phycobiont).

Lichen is widely used bioindicator as it has ability to absorb toxic materials for long time (Blett et al., 2003). It is used worldwide to determine the air quality and detect heavy metal pollution in the environment. According to Conti and Cecchetti (2001), lichen is a good bioaccumulator as it fits all the criteria which are accumulating the pollutants without being killed by the levels of toxic materials; have a wide geographical distribution; abundance, sedentary, or of scarce mobility, as well as being representative of the collection area; and have equal contaminant level with the surrounding environment in every site studied and under any condition.

Lichens are also forage for some species of animals, especially during winter. For instance, the northern species of deer known as caribou or reindeer (*Rangifer tarandus*) consumes *Cladina* sp. lichen. Lichens are also used as antibiotic as it have high antimicrobial property and antioxidant content. Lichen plays significant role in the ecological system and provide some benefits to human. Thus, more research is needed to discover its potential and provide data to initiate conservation actions to take place.

1.2 Research Problem

In Malaysia, lichens are abundant as a biomass source. However, they are always been left unnoticed and unexplored. It may be due to the lack of expertise and knowledge about the valuable properties of lichens and its fascinating functions in ecological system. Although not new in term of research, the lichenology studies in Malaysia are still in its infancy. Mainstream biotechnologists still cater for the other common plants and microorganisms. Due to this, Malaysia still depends on the expertise of lichenologists to collect, identify and extract its chemical properties for use.

Since 1866, lichens have been used as an indicator for the air quality (Nylander 1866; Kricke and Loppi 2002; Conti and Cecchetti 2001). The capability of lichens to absorb toxic materials such as sulphur dioxide (SO₂), fluorine (F₂) and nitrogen dioxide gas

(NO₂) into the thallus system for long periods of time has made the lichens an excellent indicator for air quality (Samsudin et al., 2012). Moreover, the rise in human population resulted in the rise for the demand for energy and resources for industry as well as an increase in agricultural activities to supply more food. In industrial areas, fossil fuels continuously release byproducts such as sulphur dioxide (SO₂) and nitrogen dioxide (NO₂) causing air pollution (Godinho et al., 2008). The release of pollutants from motor vehicles also contributes to air pollution in urban areas. Examples of the pollutants are NO, NO₂, CO, CO₂, poly-aromatic hydrocarbons (PAH) and heavy metals such as Pb (Larsen et al., 2007).

In Gunung Jerai area, the ecotourism concept that has been promoted by the government to encourage tourists to visit Gunung Jerai may risk the air quality in that area. According to Taher et al. (2014), one of the most popular mountains among hikers is Gunung Jerai. Due to this, the number of people visited Gunung Jerai kept increasing. With all the possible risk of human disturbance, and overcrowded of tourists of Gunung Jerai, a study to determine the air quality of Gunung Jerai needed in order to overcome or reduce the risk. This study is designed to determine the air quality of Gunung Jerai by using Index of Atmospheric Pressure (IAP). However, this study is also conducted at the nearest adjacent city to Gunung Jerai which is Sungai Petani in order to obtain the IAP score for comparison.

Lichens are very abundant in the Malaysia ecosystem. However, due to the lack of knowledge on lichenology, the potential functions of lichens are still questionable. However, some of the studies in oversea shown that lichens have potentially healthy properties such as high phenolic content and high antioxidant content (Fernández-Moriano, Gómez-Serranillos, and Crespo, 2015; Kosanić et al., 2015). According to systematic review by Fernández-Moriano, Gómez-Serranillos, and Crespo in 2015, consistent evidence demonstrated that lichens have high antioxidant activity due to the high phenolic content in some species of lichen. The reviewed data suggest that some lichen compounds are worthy of further investigation and better understanding of their antioxidant potentials. Hence, this study is conducted to determine the antioxidant content of selected lichen species in order to utilize the function of lichen as a natural source of antioxidant.

1.3 The Objectives of the Study

The objectives of this study are:

- a) to determine the lichen diversity of Gunung Jerai and Sungai Petani using Shannon Diversity Index
- b) to assess and compare the air pollution of forested site, Gunung Jerai and urban site, Sungai Petani using Index of Atmospheric Pressure (IAP) and
- c) to evaluate the antioxidant activity in selected lichen species.

1.4 Research Hypotheses

The hypotheses of this study are as follows :-

- a) Lichen diversity will increase as the altitude increase.
- b) The higher the lichen diversity, the higher the IAP score.
- c) The highest antioxidant content in lichen is from *Ramalina* lichen.

1.5 Significance of the Study

In Malaysia, the exposure of lichenology studies are very limited because of limited scientific in lichenology. Although lichens can be found substantially in Malaysia surroundings, lichens are still a rare or uncommon compared to fungi and alga. Lichens potentials are immense and have yet to be used in applications in Malaysia. Lichens have many uses such as various types of dyes, perfumes and aromatherapy products, main ingredient for Indian condiments and reindeer food. More data are needed on diversity of lichens in Malaysia to ascertain the potential applications of lichens in Malaysia. Studies on lichenology in Malaysia are needed to promote the importance of lichen and its uses to the public. This study can provide more knowledge about lichen in Malaysia and promote it's function as well.

Lichens are known as bioindicators worldwide, and thus it is used as a method to determine the air quality of spesific places using the lichen mapping method. For example, a group of lichen species was successfully selected to monitor air quality and ecological integrity at Kejimkujik National Park and National Historic Site in Nova Scotia, Canada (McMullin et al., 2017). In Malaysia, there are limited studies about lichen mapping in Malaysia. One of the studies was conducted at Universiti Kebangsaan Malaysia (UKM) to determine the air quality of the campus (Samsudin et al., 2012).

In our study, lichen diversity will be used as well as using another potential method to determine the air quality using Index of Atmospheric Pressure (IAP). This study also compare between rural and urban area to validate the use of lichen as suitable bioindicator to determine air quality in Malaysia.

Moreover, this study will provide useful data about lichen diversity of Gunung Jerai to give information about the conservation status of lichen. According to IUCN (2019), more lichen species is listed under critically endangered species because they are poorly represented in conservation efforts in the world including United States. In Malaysia, even less attention was given to conserve lichen. Thus, this study is very important as it can provide scientific data to alert related authorities about lichen extinction status and initiate action and movement to protect our valuable lichen and better management plan for conservation can be constructed using available data.

On the other hand, this study can also discover another potential of lichen in terms of health because lots of evidence show that it is a good antioxidant source and have anticancer properties. According to Katalinic et al., (2006), *Cetraria sp.* of lichen have high antioxidant content which is comparable with vitamin C and 69 other medicinal plants. In this study, selected lichen from Gunung Jerai will be analysed to determine their antioxidant content. Thus, this study will provide substantial evidences to support the usage of lichen as medicinal plant or as antioxidant source of plant where may prevent the waste of lichen function.

1.6 Limitation of the Study

The limitation of this study is only one trail was used as sampling site for Gunung Jerai because of time constraint. There might be other rare species of lichen that are developing outside the trail area that were not sampled.

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LIST OF PUBLICATIONS

Journal

Lichens diversity and index of atmospheric purity of selected sites in Kedah, Malaysia (2019). Nur Syuhada Rosli, Shahrizim Zulkifly, Nur Amalina Zaharudin, Nur Najwa Fariahah Mat Lazim, Afni Ali and Rusea Go. *Malayan Nature Journal* 71(4) 467-473.

Proceedings

Preliminary study on antioxidant activity and total flavonoid content in four Malaysian lichens. NS Rosli, S Zulkifly, NA Zaharudin, NNF Mat Lazim and R Go. ISIMBIOMAS 2019, 24-25 August 2019, Colmar Tropicale, Pahang, Malaysia.

The comparison between index of atmospheric purity (iap) of a forested and open sites in Kedah, Malaysia. Nur Syuhada Rosli, Nur Amalina Zaharudin and Shahrizim Zulkifly. Fundamental Science Congress 2018, 23-24 October 2018, RHR Hotel@UNITEN, Kajang, Selangor, Malaysia.

Canonical correspondence analysis of freshwater algae in a north lake of Ayer Hitam Forest Reserve, Puchong, Selangor. Nurul Najwa Fariahah Mat Lazim, Shahrizim Zulkifly, Intan Farhana Uyob and Nursyuhada Rosli. I-SIMBIOMAS 2019, 24-25 August 2019, Colmar Tropicale, Pahang, Malaysia.



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