



UNIVERSITI PUTRA MALAYSIA

***ANTI-TUMOR PROMOTING ACTIVITY OF *Dicranopteris linearis**
(Burm.f.) UNDERW. LEAF METHANOL EXTRACT IN A TWO-STAGE
MOUSE SKIN CARCINOGENESIS MODEL**

ROIHANAH RODZI

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By

ROIHANAH RODZI

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

April 2017

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

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April 2017

Chairman : Associate Professor Zainul Amiruddin Zakaria, PhD
Faculty : Medicine and Health Sciences

Dicranopteris linearis locally known as 'resam' has been used to treat various ailments including fever, boils, ulcers and wounds. Previous studies reported in vivo antinociceptive, anti-inflammatory, antipyretic, anti-oxidative, and anti-bacterial activity of *D. linearis*. Skin carcinogenesis incidence has been experiencing an increase worldwide. Constant exposure to physical, biological and also chemical assault on skin might lead to mild to severe skin carcinogenesis. Therefore, it is crucial to prevent it from happening. The present study was carried out to elucidate the chemopreventive potential of leaf methanol extract of *D. linearis* (MEDL) in a two-stage mouse skin carcinogenesis model since the inflammation, oxidative stress and tumor promotion pathways are interrelated. MEDL was prepared in a dose range of 30 to 300 mg/kg body weight. A total of 48 ICR female mice (6-8 weeks old) were randomly assorted into six groups. To induce skin tumor formation, a single topical application of 7,12-dimethylbenz[a]anthracene (DMBA) at 100 μ g/100 μ l was applied to the shaved dorsal region of mice, followed by repetitive administration of 1% croton oil, twice weekly for 30 weeks. Topical application of MEDL, 30 minutes prior to the croton oil application, significantly reduced the tumor incidence to 37.5% in 300 mg/kg MEDL-treated group as compared to 100.0 % in carcinogen control. The first tumor appeared at week 5 of tumor promotion period, for Group 5 (carcinogen control). Tumor in Group 1 which has been treated with MEDL at 30 mg/kg body weight appeared on week sixth. Tumor in Group 2 appeared on seventh week simultaneously with Group 4 (positive control) whereas the tumor formation latest appeared in week 12 for Group 3, which has been treated with MEDL at 300 mg/kg body weight. The tumor burden MEDL-treated groups (30, 100, and 300 mg/kg) were significantly less (25.53 \pm 3.23, 5.43 \pm 0.97, and 1.20 \pm 0.23), as compared to carcinogen control (33.70 \pm 4.25). The highest tumor volume was in carcinogen control (7.93 \pm 2.08) which is close to positive control tumor volume with the reading 7.12 \pm 1.45. Tumor volume for treated group has the reading of 18.08 \pm 4.37 mm³ (highest), 0.963 \pm 0.33 mm³ and 0.06 \pm 0.019 mm³ in 30, 100 and 300 mg/kg MEDL-treated groups, respectively. Fresh sample of skin tissue was also subjected to antioxidant assay to

determine catalase (CAT) and superoxide dismutase (SOD). As for the rest of MEDL and MEDL partition extracts, they were tested for antioxidant activity studies involving Total phenolic content (TPC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide dismutase (SOD) and oxygen radical absorbance capacity (ORAC) and anti-inflammatory study involving Lipoxygenase (LOX)-inhibiting activity and Xanthine oxidase (XO) activity. Phytochemical screening and also high-performance liquid chromatography (HPLC) were also performed to determine bioactive compound exist in the sample. The present study found that MEDL exhibited some level of inhibition of tumor promotion in dose-dependent manner with 300 mg/kg showed greatest activity, suggesting the chemopreventive potential of *Dicranopteris linearis*. In conclusion, MEDL exerted potential anti-tumor promoting activity, having the highest dose (300mg/kg) of MEDL performing the best. It is found that MEDL possessed high antioxidant activity but low in anti-inflammatory activity.



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

**AKTIVITI ANTI-TUMOR MENGGUNAKAN EKTRAK METANOL DAUN
Dicranopteris linearis (Burm.f) DALAM UJIAN DUA PERINGKAT
KARSINOGENESIS KULIT DALAM MENCIT**

Oleh

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Pengerusi : Profesor Madya Zainul Amiruddin Zakaria, PhD
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Dicranopteris linearis yang juga dikenali sebagai paku resam telah digunakan untuk merawat pelbagai penyakit, termasuk demam, bisul, ulser dan luka. Kajian sebelum ini telah melaporkan aktiviti in vivo *D. linearis* seperti anti-kesakitan, anti-radang, anti-piretik, anti-pengoksidaan dan anti-bakteria. Karsinogenesis kulit yang dialami seluruh dunia kian meningkat. Pendedahan secara fizikal, biologi dan kimia secara berpanjangan ke atas kulit akan mengakibatkan kanser kulit samada pada tahap ringan atau teruk. Oleh itu, pencegahan daripada ianya berlaku adalah penting. Kajian ini telah dijalankan untuk menentukan potensi kemopreventif ekstrak metanol daun *D. linearis* (MEDL) ke atas model karsinogenesis kulit mencit dua peringkat, memandangkan inflamasi, tekanan oksidatif and penggalakan tumor adalah saling berkaitan. MEDL telah disediakan dalam julat dos daripada 30 sehingga 300 mg/kg berat badan. Sebanyak 48 mencit betina ICR (umur 6-8 minggu) telah dibahagikan kepada enam kumpulan secara rawak. Untuk merangsang pembentukan tumor kulit, satu rawatan topikal 7,12-dimethylbenz[a]anthracene (DMBA) pada dos 100 μ g/100 μ l telah lakukan ke atas kulit dorsal mencit yang telah dicukur. Ini diikuti dengan rawatan 1% kroton oil secara berulang, dua kali seminggu selama 30 minggu. Rawatan MEDL secara topikal, 30 minit sebelum rawatan minyak kroton megurangkan insiden tumor secara signifikan kepada 37.5% bagi kumpulan rawatan MEDL pada 300mg/kg berat badan berbanding dengan 100.0% bagi kawalan karsinogen. Tempoh pedaman tumor telah meningkat daripada minggu ke-lima bagi kawalan karsinogen. Tumor bagi kumpulan 1 yang dirawat dengan 30mg/kg MEDL muncul pada minggu ke-enam manakala tumor bagi kumpulan 2 muncul pada minggu ke-tujuh seiring dengan kumpulan 4 (kawalan positif). Tempoh pedaman tumor terakhir muncul pada minggu ke 12 bagi kumpulan 3 yang dirawat dengan 300mg/kg berat badan MEDL dan terus meningkat hingga minggu ke 30. Beban tumor bagi kumpulan rawatan MEDL (30, 100 dan 300 mg/kg) telah diringankan secara signifikan (25.53 ± 3.23 , 5.43 ± 0.97 , dan 1.20 ± 0.23) berbanding dengan kawalan karsinogen (33.70 ± 4.25). Isipadu tumor mempunyai bacaan pada 18.08 ± 4.37 mm³, 0.963 ± 0.33 mm³ dan 0.06 ± 0.019 mm³

bagi kumpulan rawatan 30, 100, 300mg/kg berat badan masing-masing. Sample kulit segar diteruskan dengan ujian antioksidan bagi menentukan aktiviti katalase (CAT) and superoxide dismutase (SOD). Manakala bagi MEDL dan ekstrak pecahan PEDL, EADL dan AQDL, menjalankan ujian antioksidan melibatkan total phenolic content (TPC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide dismutase (SOD) dan oxygen radical absorbance capacity (ORAC). Bagi ujian anti-radang pula, kajian ini melibatkan Lipoxygenase (LOX)-inhibiting activity dan aktiviti Xanthine oxidase (XO). Ujian saringan fitokimia dan juga high performance liquid chromatography (HPLC) juga dijalankan bagi menentukan kompoun bioaktif yang terkandung di dalam MEDL. Kajian ini mendapati bahawa MEDL mempamerkan tahap tertentu perencatan penggalakan tumor yang berkaitan dengan dos, dengan 300 mg/kg menunjukkan aktiviti paling berkesan, mencadangkan potensi kemopreventif *Dicranopteris linearis*. Kesimpulannya, MEDL mempunyai potensi sebagai agen anti-tumor, dengan dos tertinggi iaitu (300mg/kg) MEDL menampilkan keputusan terbaik. MEDL juga mempunyai aktiviti antioksidasi yang tinggi tetapi mempunyai kadar anti radang aktiviti yang lemah.

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I certify that a Thesis Examination Committee has met on 18 April 2017 to conduct the final examination of Roihanah binti Rodzi on her thesis entitled "Anti-Tumor Promoting Activity of *Dicranopteris linearis* (Burm.f.) Underw. Leaf Methanol Extract in a Two-Stage Mouse Skin Carcinogenesis Model" 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 be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AQDL	Aqueous extract of <i>Dicranopteris linearis</i>
DMBA	7,12-Dimethylbenz(a)anthracene
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EADL	Ethyl acetate extract of <i>Dicranopteris linearis</i>
HPLC	High performance liquid chromatography
IC50	Median inhibitory concentration
LOX	Lipoxygenase
MEDL	Methanol extract of <i>Dicranopteris linearis</i>
ORAC	Oxygen radical absorbance capacity
PEDL	Petroleum ether extract of <i>Dicranopteris linearis</i>
ROS	Reactive oxygen species
SEM	Standard error mean
SOD	Superoxide dismutase
TPC	Total phenolic content
WHO	World Health Organisation
XO	Xanthine oxidase

CHAPTER 1

INTRODUCTION

Cancer remains as major cause of mortality and morbidity which also is one of the major public health problem areas in developing as well as in developed countries. Chemotherapy using antineoplastic drugs to kill rapid dividing cells is one of the commercial ways of providing treatments for cancer patients. Nevertheless, the side effects caused by these drugs have been overshadowed by its effectiveness. Its side effects include harm to the rapid dividing cells (i.e. bone marrow, digestive tract and hair follicles) which afterwards lead to immunosuppression, mucositis and hair loss (Ooi *et al.*, 2014).

There has been an increase in the incidence of both non-melanoma and melanoma skin cancers over the past decades. Non-melanoma skin cancers are between 2 and 3 million cases whereas currently there are 132, 000 melanoma skin cancers that occur every year globally (“World Health Organization. Skin Cancer, “2016). Apparently, there’s an established connection between tissue damage, inflammation and cancer development. Tumorigenesis is related to pathway activation whereas inflammation is a self-limiting process in normal conditions (Neagu *et al.*, 2016). Inflammation is triggered by something such as constant chemical exposure on the skin and some other environmental factor which in return will allow the initiation and progression of skin cancer. The major traits for tumor initiation and progression of the skin is chronic inflammation (Neagu *et al.*, 2016).

Some herbs and medicinal plants have the phytochemical constituents that contain bioactive compound which possessed anti-tumor or anti-carcinogenesis activity. These bioactive compounds are important in the cancer preventive strategy in order to inhibit, delay or even reverse carcinogenesis (Ooi *et al.*, 2014). *D. linearis* is one of the plant being studied in the laboratory. Locally known as ‘Resam’, this plant has its own hidden potential. This fern type medicinal plant has been scientifically reported to have diverse pharmacological activities.

Cancer prevention using natural products has earned accelerating attention these recent years. Due to the promising strike on human health, laboratory and clinical testing has been done extensively as an effort in identifying new chemopreventive agents. This current studies were executed to scrutinize the anti-tumor promoting activity (Rodzi *et al.*, 2013). Significantly, discovering the plant’s potential for its promising pharmacological properties will enable exploitation of the sources in preventing relatable ailments.

Problem statement

Skin cancer incidence has been experiencing a prompt increase, as one of the most common malignancies affecting human populations worldwide. As humans grows, the skin may experience constant exposure to complex agents, such as physical (sun rays) and biological assaults (microbes and or allergens) (Neagu *et al.*, 2016). Not to mentioned, skin also is exposed to chemicals indirectly through daily unauthorized harmful skin care products consumptions, side effects of existing drugs or working environment which may require handling of chemicals. These circumstances may lead to skin carcinogenesis in the long run. In this modern era, it is undeniable there's advanced new modern medications and approach in treating diseases. Unfortunately, there's also several impediments to the public, such as expensive medications and procedures, side effects caused by the medicine, current medical conditions of the patients that forbids them for taking certain medicine, and also lack of drugs availability. Consequently, the search of alternative medicinal drug in preventing and hopefully can reduce skin carcinogenesis is extremely recommended. Therefore, extensive studies have been conducted on this natural product to discover its potential of the anti-tumor promoting activity of *D.linearis*. Previous studies reported that *D.linearis* possesses anti-inflammatory and antioxidant activity which is relevant to anti-tumor promoting activity. In this study, it is expected to measure the anti-tumor promoting activity of *D.linearis* towards the two-stage mouse skin carcinogenesis model.

Justification on studying the anti-tumor potential of *D. linearis*

Modern drugs that are available nowadays might have unknown side effects especially in the long run. While some patients with certain health conditions are unable to consume certain drugs due to its unavoidable side effects which might put them at risk, some other patients might be having trouble getting the drugs due to a very high cost of medication and lack of drug availability. Therefore, several alternatives must be explored to find the remedy in order to prevent, reduce, treat or even cure the ailments. Alternatives recommended must be safe to consume, very less to no side effects in short term and long term and also not to mention it must prevails its effectiveness in either preventing, reducing, or curing the illness. Apart from the side effects mentioned, these alternatives also should come in a cheaper price and widely available.

Natural products such as medicinal plants are one of the better alternatives to these modern drugs. Medicinal plants can be widely found and through extensive studies, it indicates that phytochemicals content in the particular medicinal plants can be utilised in treating many health problems. *D. linearis* for instance, are widely abundant and have its own potential based on the traditional uses of this plants. Traditionally, this plant was used to reduce body temperature and control fever, treat asthma, treating external wounds ulcers and broils and also getting rid of intestinal worms. This has shown that this particular plant has its own potential or maybe can offer more to health care industries via thorough research. This study was aimed to discover the potential of *D. linearis* leaves extract in exhibiting its chemopreventive activity. Previous

studies were reported that *D. linearis* have anti-oxidants and anti-inflammatory activity thus making it being relevant to chemopreventive studies. Promising anti-oxidants as well as anti-inflammatory activities has triggered this study knowing that there are connections between the inflammatory, oxidative and cancer processes. The capability to inhibit any of the processes mentioned will undoubtedly lead to the inhibition of the others. Hence, this study is expected to unveil the *D. linearis* potentials for this anti-tumor promoting activity on two-stage mouse skin carcinogenesis model.

Hyphothesis

Methanolic extract of *D. linearis* leaves exhibits anti-tumor promoting activity in this DMBA/Croton oil induced two stage skin carcinogenesis model.

General objectives

To determine anti-tumor promoting effects of methanolic extract of *Dicranopteris linearis* leaves (MEDL) on DMBA/Croton Oil-induced mouse skin carcinogenesis.

Specific objectives

- To determine the effective dose range of MEDL in suppressing tumor growth on DMBA/Croton Oil-induced mouse skin carcinogenesis.
- To examine the presence of anti-oxidant and anti-inflammatory activities of the extracts involved in suppressing tumor growth.
- To screen for the bioactive compounds, present in MEDL using high performance liquid chromatography (HPLC).
- To examine the involvement of antioxidant activities of the tissue as part of the skin carcinogenesis pathway.

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