



**UNIVERSITI PUTRA MALAYSIA**

**ANTIOXIDANT, ANTIPROLIFERATIVE AND  
ANTIMICROBIAL PROPERTIES OF LEAF EXTRACTS OF PERESKIA  
GRANDIFOLIA, PERESKIA BLEO AND POLYGONUM ODORATUM  
LOUR**

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Science**

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fulfilment of the requirement for the degree of Master of Science

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Malaysia is one of the Asian countries which are endowed with highly diverse biological resources. Indeed, quite high percentage of flora available in this country is believed to have medicinal and nutritional values. Even though a few species such as “Kacip Fatimah”, “Tongkat Ali” and “Misai Kucing”, just to name a few has undergone scientific research, but they are many more need thorough investigation. Studies on the antioxidant, antiproliferative and antimicrobial properties of ethanol leaf extracts of *Pereskia bleo*, *Pereskia grandifolia* and *Polygonum odoratum* were undertaken. DPPH assay was carried out to measure the capacity of the extracts to scavenge free radicals, whereas the inhibitions of lipid peroxidation by the

extracts were done using  $\beta$ -carotene bleaching method. The phenolics content was quantified using Folin Ciocalteau reagent and the correlation between total phenolics content and antioxidant activity was tested. Antiproliferative property of the extracts were assessed using MTT assay on different cancer cell lines, namely CaOV<sub>3</sub>, HeLa, HepG2 and MDA-MB231, while the antimicrobial property of the extracts were screened using disc diffusion assay. Determination of vitamin A, C, E and mineral contents were also carried out. From both antioxidant assays, these extract showed high antioxidant activities which could be attributed to the occurrence of phenolics. Result obtained from MTT assay showed that the proliferations of HeLa and CaOV<sub>3</sub> cells were effectively inhibited by the extracts. However, a poor antimicrobial activity was exhibited on both Gram-positive and Gram-negative bacteria as well as *Candida albicans* strains. In conclusion, all plant extracts demonstrated high antioxidative and antiproliferative properties which could be attributed to their phytochemical contents.

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

**CIRI ANTIOKSIDAN, ANTIPROLIFERASI DAN ANTIMIKROBIAL BAGI  
EKSTRAK DAUN *PERESKIA GRANDIFOLIA*, *PERESKIA BLEO* DAN  
*POLYGONUM ODORATUM LOUR***

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Malaysia merupakan salah sebuah negara Asian yang dianugerahkan dengan kekayaan khazanah semulajadi. Terdapat pelbagai spesis flora yang terdapat di negara ini dipercayai mempunyai nilai perubatan dan pemakanan. Meskipun terdapat beberapa spesis seperti Kacip Fatimah, Tongkat Ali dan Misai Kucing yang telah menjalani kajian saintifik, namun masih banyak lagi yang memerlukan kajian secara mendalam. Kajian tentang ciri-ciri antioksidan, antiproliferasi dan antimikrob telah dijalankan ke atas ekstrak etanol daun *Pereskia bleo*, *Pereskia grandifolia* dan *Polygonum odoratum*. Asai "DPPH radical scavenging" telah dilakukan untuk mengukur kapasiti ekstrak untuk memerangkap radikal bebas, manakala kesan perencatan ekstrak ke atas peroksidasi lipid telah diuji melalui asai

" $\beta$ -carotene bleaching". Kandungan fenol ditentukan menggunakan reagen folin ciocalteau dan korelasi di antara jumlah kandungan fenol dan aktiviti antioksidan bagi setiap ekstrak tumbuhan kajian ditentukan. Penentuan ciri antiproliferasi juga dijalankan melalui asai MTT ke atas beberapa jenis titisan sel kanser iaitu, CaOV<sub>3</sub>, HeLa, HepG2 dan MDA-MB231. Ciri antimikrob pula diuji menggunakan asai "Disc diffusion". Penentuan kandungan vitamin A, C dan E serta mineral juga telah dilakukan. Hasil dari kedua-dua asai antioksidan, menunjukkan terdapat aktiviti antioksidan yang tinggi dalam ekstrak tumbuhan kajian, yang mana berkemungkinan disebabkan oleh kehadiran bahan fenolik. Asai MTT pula menunjukkan kesan antiproliferasi ekstrak terhadap dua jenis titisan sel kanser, iaitu CaOV<sub>3</sub> dan HeLa. Walaubagaimanapun, aktiviti antimikrob yang lemah ditunjukkan oleh ketiga-tiga jenis ekstrak tersebut ke atas semua strain bakteria Gram-positif, Gram-negatif dan juga *Candida albicans* yang diuji. Kesimpulan dari kajian ini adalah, kesemua ekstrak kajian mempunyai ciri antioksidan dan antiproliferasi yang mungkin disumbangkan oleh bahan fitokimia yang terdapat dalam tumbuhan tersebut.

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## LIST OF ABBREVIATION

HeLa	Cervical cancer cell lines
CaOV <sub>3</sub>	Ovarian cancer cell lines
HepG2	Hepatic cancer cell lines
MDA-MB231	Non hormone dependent breast cancer cell lines
Chang liver	Hepatic cell lines
MTT	Microculture tetrazolium assay (3-[4,5-dimethylthiazol -2-yl]-2,5-diphenyltetrazoliumbromide)
DMEM	Dulbecco's Modification of Eagle's Medium
EMEM	Earl's Modification of Eagle's Medium
RPMI 1640	Roswell Park Memorial Institute



## CHAPTER 1

### INTRODUCTION

Substances that have been proved to have medicinal properties are considered as pharmaceuticals. To date, numerous researches on pharmaceuticals have been scientifically conducted world wide. These encompass the drug composition and properties, interactions, toxicology, therapy and medical applications of natural products especially from plant sources.

As one of the most rapidly developing country, Malaysia has shown advancement in scientific researches in this field. This might be due to the enhancement of pharmaceutical industry in this country. The Industry, Investment, Trade and Productivity Performance Report of first quarter of year 2005 reported by the Ministry of International Trade and Industry Malaysia (2005) showed that pharmaceutical industry posted a significant growth of 27.5 per cent in 2005 compared with 2004. This can be observed in Table 1 (Appendix 1), which shows the production index by industrial sector in Malaysia for the first quarter of the year 2005. Thus, research in this field becomes even more crucial.

Malaysia is one of the Asian countries which are endowed with highly diverse biological resources. In deed, a quite high percentage of the flora

available in Malaysia is believed to have medicinal and nutritional values, yet needs more scientific investigation. Even though a few species of flora in Malaysia such as 'Kacip fatimah', 'Tongkat ali' and 'Misai kucing' just to name a few, has undergone scientific research, but there are many more needs to be investigated scientifically.

Presently, the superiority of medicinal plants or traditional medicine to modern drugs was generally accepted among our society. This can be observed based on the significant growth in the pharmaceutical industry in recent years as mentioned earlier. Besides, there was an increase of demand on natural products, such as health supplements and cosmetics among consumers. Scientific evaluations are very important to avoid side effects that might be resulted from the natural products used. Issues regarding the efficacies, safe dosage, and proper application are required to be taken into consideration in order to avoid any over expectation and abuse.



Figure 1.1: *P. bleo* (DC.) Kunth (Magnification: 300 x 198 pixels)

*Pereskia bleo* belongs to the family of cactacea. This plant is native to tropical America (IPNI, 2005). Other names for this plant are *Pereskia cruenta*, *Pereskia panamensis*, *Rhodocactus corrugatus*, *Pereskia corrugate*, *Rhodocactus bleo* and *Cactus bleo* (Wikipedia, 2006). It is popular in Chinese community as "Pokok 1001 Khasiat", and believed to have anticancer properties, where their young leaves are eaten as vegetable. Pereskias, the only cactus with leaf, stay true to its name with large strong colored orange flowers that resemble a delicate rose. All along its stem are found seven sharp needles. This gives it the characteristic of a cactus. Contrary to other cacti species, this plant is not succulent and the cuttings need a moist condition for optimum growth. This plant is known as "Seven needles" or "Jarum Tujuh Bilah".





Figure 1.2: *P. grandifolia* (Haw.) Kunth (Magnification: 216 x 182 pixels)

*Pereskia grandifolia* is a scandent shrub which is characterized by large pink flowers with a yellow centre. Also belongs to cactacea family (IPNI, 2005), this pereskia is reported to be more tolerant to cold temperature compared to *P. bleo*. Other names of *P. grandifolia* are *Rhodocactus grandifolius*, *Cactus grandifolius*, *Pereskia grandiflora*, *Pereskia tampicaria* and also *Rhodocactus tampicanus*. The origin of this plant is from Brazil (Espírito Santo and Minas Gerais). However, it has been reported that this plant is also found in Mexico (*Pereskia tampicana*). It might also escaped cultivation, as it was available in abundance in Malaysia. *P. grandifolia* has the same external appearance as *P.bleo*, thus it is also “Jarum tujuh bilah”. The contrast between these two species is the colour of their flowers.



Figure 1.3: *P. odoratum* Lour. (Magnification: 350 x 263 pixels)

Another plant studied was *Polygonum odoratum* Lour. *P. odoratum* (Polygonaceae) (IPNI, 2005) or its local name, Kesom is a culinary herb widely used in Southeast Asian cooking and for the production of Kesom oil, a potential source of natural aliphatic aldehydes. It has been reported that the essential oil of *P. odoratum* contains long-chain aldehydes. The aldehydes are decanal (28%), dodecanal (44%), decanol (11%) and sesquiterpenes ( $\alpha$ -humulene,  $\beta$ -caryophyllene) which account for about 15% of the essential oil (Hunter *et al.*, 1997).

## **Problem Statement**

In normal condition, cells in our body are dividing with the demand for new cells. However, there are times when cells of certain part of the body proliferate without control, and results in the development of cancerous tissue mass or tumor. If the tissues were formed from normal cells, they are called benign cell. In contrast, the tissues formed from abnormal cells are called malignant cells, cells that are responsible to cause cancer (Scott, 1979).

Cancer is a public health problem worldwide. Cancer can attack anybody, no matter who its victims are. It affects all people: the young and old, the rich and poor, men, women and children. Cancer is the uncontrolled growth and spread of cells that may affect almost any tissue of the body. Lung, colorectal and stomach cancer are among the five most common cancers in the world for both men and women. Among men, lung and stomach cancer are the most common cancers worldwide. For women, the most common cancers are breast and cervical cancer (WHO, 2006). More than 11 million people are diagnosed with cancer every year. It is estimated that there will be 16 million new cases every year by 2020. Cancer causes 7 million deaths every year and 12.5% of deaths worldwide (WHO, 2006).

The problem of cancer in Malaysia is a growing one as it was reported that the annual incidence of cancer in Malaysia has been estimated to be 30 000 (Lim and Lim 1993), while the prevalence of cancer was estimated to be approximately 90 000. In 1999, the prevalence of cancer was estimated to reach 230 cases in 100,000 Malaysians (Malaysia Ministry of Health, 1999). As example, cancer of the cervix which is the second most common cancer among females in Malaysia showed incidence rate of 11.6 per 100,000 populations, with the age standardized rate of 16.2 per 100,000 (Nor Hayati, 2002).

In fact, the incidence of cancer is expected to rise with an increase in aging population. This situation is expected as the elderly are most susceptible to many cancer risk factors. Karim (1997) has reported that the proportion aged more than 60 years was 4.6% in 1957. This has increased to 5.7% in 1990 and is projected to be 9.8% in 2020 (Karim, 1997). Cancer among children is also increasing where the incidence rate of cancer for this target group was reported to be 77.4 in every million children aged below fifteen years of age (Malaysia Ministry of Health, 1999).

### **Significance of the Study**

Nowadays, concerns over health are gaining attention across the Malaysian community. It can be observed through increased demand for health food



products. In fact, the public now have become more aware and conscious of the importance of diet and foods for their health.

It has been well documented that dietary factors might play a vital role in health protection and prevention of getting any chronic diseases by modulating the inner mechanism through several biochemical pathways. One of the examples is the development of cancer. Therefore, further research has to be carried out to identify specific components which are present in various species of plant such as fruits, vegetables, leaves and many more which exhibited high antioxidant, antiproliferation and several other beneficial properties.

Preliminary studies indicated that plants, generally represented by fruits and vegetables contain phytochemical components. Phytochemicals are chemical substance that can be abundantly found in plants. They are believed to act as anticancer agent. It has been reported that the effectiveness of the antioxidant and antiinflammatory properties relies beneath the phytochemical inhibit the proliferation of tumor from the initiation stage, propagation and the progression stage (Huang, 1992).

Epidemiological study showed that the frequency and high intake of vegetables and fruits correlate to the cancer incidence. The increase of ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, vitamin A and other phytochemical

levels, due to daily intake were highly potential in reducing the incidence of cancer (Huang and Ferraro, 1992).

Realizing the importance and benefits of its findings to nutrition and health, this study was carried out to reveal the antioxidant activity, antiproliferative and antimicrobial properties of these plants. This study aimed to contribute more scientific findings on the plants investigated. *Pereskia bleo*, *Pereskia grandifolia* and *Polygonum odoratum* were commonly found in Malaysia. The leaves of *P. bleo* and *P. grandifolia* were believed to have medicinal properties, where they are eaten as vegetable. As there was still lack of scientific findings available regarding these plants, this study will enhance the awareness and knowledge of the importance of natural remedies and also contributes more scientific data on it.