UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITY OF SILVER COMET (*GLOBBA PENDULA*)

MAULIDIANI

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CHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITY OF SILVER COMET (*GLOBBA PENDULA*)

By

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

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CHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITY OF SILVER COMET *(GLOBBA PENDULA)*

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December 2007

Chairman : Professor Md. Nordin Hj. Lajis, PhD

Institute : Bioscience

In a continuation of our chemical and biological investigations on Zingiberaceae species, we have now studied *Globba pendula*, a less common ginger plant found in Peninsula Malaysia. This plant has been used traditionally as a protective medicine after childbirth and for treating stomach complaints. No biological activity and chemical constituents have been reported on this species so far.

Five of eight extracts including dichloromethane (rhizome and leaf), ethyl acetate (rhizome and leaf), and hexane (leaf) from the sequential extraction of *Globba pendula* were evaluated for cytotoxic activity. They exhibited cytotoxicity against MCF-7 cells (human breast cancer) with IC_{50} values ranging from 19.5 to 37.0 µg/ml. The statistical analysis of variance (ANOVA) showed that there were no significant ($P<0.05$) difference of the cytotoxic activity among the extracts. Phytochemical studies on the rhizomes and leaves of *Globba pendula* resulted in the isolation of two new compounds. They are 16-oxo-(8)17-12-labdadien-15,11-olide **(92)** and

benzofuran-2-carboxaldehyde **(96)**, along with seven known compounds: 4-hydroxy-3-methoxybenzoic acid **(69)**, *β*-sitosterol **(73)**, *β*-sitosteryl-*β*-D-glucopyranoside **(90)**, 7α-hydroxysitosterol **(91)**, 3,14,19-trihydroxy-8(17),12-labdadien-16,15-olide **(93),** 4-hydroxy-3-methoxybenzaldehyde **(94)**, and 2(*3H*)-benzoxazolone **(95)**. The structures of isolated compounds were established based on spectroscopic data and comparison with the literature. The compound 3,14,19-trihydroxy-8(17),12-labdadien-16,15-olide **(94)** has demonstrated strong cytotoxic properties towards a panel of cancer cell lines (MCF-7, PC-3, and H-460) with the IC50 values of 7.9, 8.7, and 9.0 µM, respectively.

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

KANDUNGAN KIMIA DAN AKTIVITI SITOTOKSIK HALIA ROYAN (*GLOBBA PENDULA*)

Oleh

MAULIDIANI

December 2007

Pengerusi: Profesor Md. Nordin Hj. Lajis, PhD

Institut: Biosains

Demi melanjutkan penyelidikan kami ke atas aspek kimia dan biologi daripada spesies Zingiberaceae, kami telah memilih *Globba pendula*, spesies yang tidak kerap ditemui di Malaysia. Menurut kajian etnomedik, tumbuhan *Globba pendula* digunakan sebagai ramuan selepas bersalin dan untuk merawat sakit perut. Setakat ini tiada laporan kajian kimia mahupun biologi ke atas spesies tersebut.

Lima daripada lapan ekstrak tumbuhan *Globba pendula* yang didapati daripada pengekstrakan secara rendaman sejuk iaitu ekstrak diklorometana (akar dan daun), etil asetat (akar dan daun), dan heksana (daun) menunjukkan aktiviti sitotoksik terhadap sel kanser payudara (MCF-7) dengan IC_{50} 19.5-37.0 µg/ml. Ujian statistic yang menggunakan analisis ANOVA menunjukkan tiada perbezaan yang signifikan ($P<0.05$) terhadap aktiviti sitotoksik di antara ekstrak-ekstrak. Kajian fitokimia akar dan daun *Globba pendula* berjaya memencilkan dua sebatian baru iaitu 16-okso-

(8)17-12-labdadiena-15,11-olida **(92)** dan benzofuran-2-karboksaldehid **(96)**. Di samping itu turut diperolehi 4-hidroksi-3-metoksibenzoik asid **(69)**, *β*-sitosterol **(73)**, *β*-sitosteril-*β*-D-glukopiranosid **(90)**, 7*α*-hidroksitosterol **(91)**, 3,14,19-trihidroksi-8(17),12-labdadiena-16,15-olida **(93)**, 4-hidroksi-3-metoksibenzaldehid **(94)**, dan 2(*3H*)-benzoxazolon **(95)**. Struktur semua sebatian dikenal pasti berdasarkan kaedah spektroskopi dan perbandingan dengan literatur. 3,14,19-Trihidroksi-8(17),12-labdadiena-16,15-olida **(93)** didapati sitotoksik ke atas panel sel-sel kanser, antaranya sel kanser payudara (MCF-7), sel kanser prostat (PC-3), dan sel kanser peparu (H-460), dengan nilai IC_{50} masing-masing 7.9, 8.7, dan 9.0 µM.

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I certify that Examination Committee met on 27 December 2007 to conduct the final examination of **Maulidiani** on her **Master of Science** thesis entitled "**Chemical constituents and Cytotoxic Activity of Silver Comet *(Globba pendula)***" in accordance with Universiti Pertanian Malaysia Malaysia (Higher Degree) Act Regulations 1980 and Universiti Pertanian Malaysia Regulations 1981. The Committee recommends that the candidates be awarded the relevant degree. Members of the Examination Committee are follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follow:

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Date: 21 February 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree at UPM or other institutions.

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MAULIDIANI

Date : 19 February 2008

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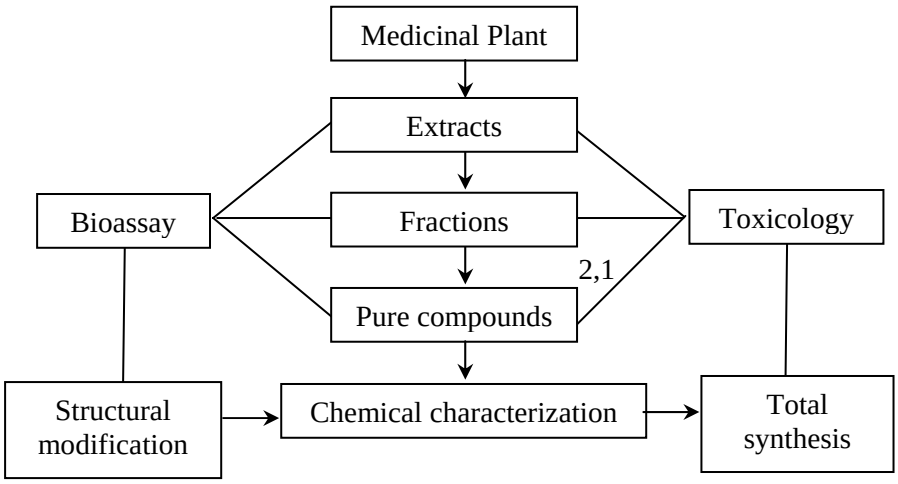
δ	Chemical shift in ppm
APCI-MS	Atmospheric Pressure Chemical Ionization Mass Spectroscopy
^{0}C	Degree in Celsius
bp	Boiling point
br	Broad
^{13}C	Carbon-13
COSY	Correlation Spectroscopy
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
dt	Doublet of triplets
DMSO	Dimethylsulfoxide
eV	Electron volt
FT-IR	Fourier Transform Infra-Red
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single-Quantum Coherence
EIMS	Electron Impact Mass Spectrum
Hz	Hertz
IC_{50}	Inhibition concentration at 50 percent
IR	Infrared

J	Coupling Constant in Hz
Lit.	Literature
m	Multiplet
m/z	Mass per charge
MHz	Megahertz
m.p.	Melting point
MS	Mass spectrum/Mass Spectroscopy
nm	Nanometer
NMR	Nuclear Magnetic Resonance
R_f	Retention Factor
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
UV	Ultraviolet
UV/VIS	Ultraviolet/visible
IC	Inhibition concentration
VLC	Vacuum Liquid Chromatography

CHAPTER 1

INTRODUCTION

Plants have been used for thousands of years to treat man's illnesses and injuries. Despite the tremendous advances made by modern medical practices their contribution are still important until today (Soepadmo, 1998). The World Health Organization (WHO) estimated that as much as 80% of the world population relies on the use of various forms of traditional (herbal) medicine for its primary healthcare (Cragg *et al.*, 1999; Narins, 2000). An impressive number of modern drugs have been isolated from natural sources based on their use in traditional medicines. Thus, the best way to find new applications of plant derived drugs would seem to be the combination of local knowledge and the modern research techniques available today. General method for obtaining active substances from plants is described in Scheme 1.1 below.



1. Fractionating process
2. Purification

Scheme 1.1: Method for obtaining active substances from plants (Rates, 2001)

It is estimated that among estimated 250000-500000 identified plant species, only a small percentage of them have been investigated phytochemically and, even a smaller percentage, in terms of their pharmacological studies (Payne *et al.*, 1991). This was further supported by Cordell (2003), who reported that less than 20% of all plant species have been evaluated chemically or biologically. Therefore, the potential uses of higher plants as a source of drugs still need to be explored.

Malaysia is considered among the plant biodiversity hot spots of the world because of its tropical rainforests, which exhibits diversity and richness not only at the community level but also at the family and genus levels. It is estimated that about 10,000 species are present in Peninsular Malaysia with at least 1158 of them reported to have medicinal value. Apart from treating common illness such as headaches, coughs and colds, some species are also used for infectious diseases like malaria and cholera (Soepadmo, 1998).

The ginger family, Zingiberaceae, is one of characteristic flora extensively found in Malaysia. For more than two decades there has been an increasing interest in the study of the plant family Zingiberaceae. Some of the popular species from the Zingiberaceae family have been studied for its chemical constituents, resulting in the isolation of numerous compounds, some of which may have potential properties as source of drugs. Below are some of the species from the Zingiberaceae family that have been studied in the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia (Table 1.1).

Table 1.1: Chemical and biological studies of some Zingiberaceae species

Species	Compound(s) isolated	Biological activities
Hedychium thyrsiforme (Jasril *et al.*, 2002)	Flavonoids	Antioxidant, anticancer
Alpinia rafflesiana (Mohamad *et al.*, 2004)	Diarylheptanoid, flavonoids	Antioxidant, antimicrobial, antiinflammatory
Alpinia zerumbet (Mohamad, 2005)	Diarylheptanoid, flavonoids	Anticancer, antioxidant
Etlingera elatior (Mohamad *et al.*, 2005)	Labdane diterpene, diarylheptanoids	Anticancer, antioxidant
Curcuma mangga (Abas *et al.*, 2005)	Curcumanggoside, labdane diterpenes, diarylheptanoids	Anticancer, antioxidant
Curcuma xanthorrhiza (Ruslay *et al.*, 2007)	Diarylheptanoids	Antioxidant
Zingiber zerumbet (Ruslay *et al.*, 2007)	Zerumbone, kaempferol glucosides	Antioxidant

The genus *Globba* is a member of the Zingiberaceae family. About one hundred species are recognized and most of them have their distribution within the northern monsoon area, from the eastern Himalayas through Burma and Thailand to Laos, Cambodia and Vietnam (Larsen, 1972). Unfortunately, very little information is known regarding their chemicals and biological properties. A species of the genus found throughout Peninsula Malaysia is *Globba pendula.* Ethnomedical reports mentioned *Globba pendula* as a herb used traditionally as protective medicine after childbirth (Burkill, 1966).

Cancer has become one of the important diseases to mankind. Over ten million new cases of cancer (all sites excluding non-melanoma skin), with over six million deaths, were estimated in the year 2000 (Parkin, 2001). Cancer is a group of many related diseases in which abnormal cells grow out of control and spread. The WHO has estimated that about 15 million new cases of cancer will develop in the year 2020, as compared to 10 million cases a year in the late 1990s. The reasons include increased smoking habit in the developing nations, unhealthy diets, and more people are living to old age when cancer risk is higher. The WHO also predicted that the prevalence of cancer cases will increase in the first 25 years of the twenty-first century in developing nations (Izenberg, 2000).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, many new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman *et al.*, 2003; Butler, 2004). On the basis of the reasons mentioned, we are interested to search for new bioactive compounds from *Globba pendula* with cytotoxic activity against cancer cell-lines.

The main objectives of this study are:

1. To isolate and identify the chemical constituents in the leaves and rhizomes of *Globba pendula*.
2. To determine the cytotoxic activity of the extract and isolated compounds from *Globba pendula*, against cancer cell-lines