SCREENING OF ANTIPROLIFERATIVE ACTIVITY OF *Artocarpus* sp. TOWARDS HeLa CELL LINE AND CaOv-3 CELL LINE

By

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CERTIFICATE

This is to certify that the dissertation entittled "Screening of antiproliferative activity of *Artocarpus* sp. towards Hela cell lines and CaOv-3 cell lines" is the bonafide record of research work done by Ms Khairun Nisa Ibrahim Asri during the period from July 2009 to October 2009 under my supervision

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LIST OF ABRREVIATIONS

AIDS	Autoimmune deficiency syndrome
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
Ag	Antigen
CARDS	Caspase recruitment domains
Cm	Centimeter
cm^2	Square centimeter
CO_2	Carbon dioxide
dATP	Deoxyadenosine triphosphate
DD	Death domain
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FADD	Fas-associated death domains
FasL	Fas ligand
FBS	Fetal bovine serum
G	Gram
HCL	Hydrochloric acid
HPV	Human papilloma virus

Hr	Hour
IC_{50}	Median concentration that cause 50% inhibition
IUPAC	International Union of Pure and Applied Chemistry
KCl	Potassium chloride
M	Meter
M	Molar
MDCK	Madin- Darby Canine Kidney
Mg	Miligram
Mg/mL	Miligram per milileter
mL	Mililiter
M	Mole
MBA	Methylene Blue Assay
N	Normal
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NCI	National Cancer Institute
NCR	National Cancer Registration
OD	Optical density
PBS	Phosphate buffer saline
Rpm	Round per minutes
TNF	Tumor necrosis factor
TNF-R1	Tumor necrosis factor receptor 1
TRADD	TNF-R1-associated death domains
WHO	World Health Organization

µg/mL	Microgram per mililiter
µL	Microliter
µm	Micrometer
v/v	Volume per volume
^{0}C	Degree celcius
%	Percentage

SCREENING OF ANTIPROLIFERATIVE ACTIVITY OF *Artocarpus* sp. TOWARDS HeLa CELL LINES AND CaOv-3 CELL LINES

ABSTRACT

Artocarpus sp. has been used as traditional folk medicine in Southeast Asia for treating ulcer, abscess, and diarrhea. This species also been reported to be used for treatment of inflammation, and malarial fever. In this study, three *Artocarpus* sp. extract were used to treat two types of cancer cell lines that are cervical cancer cell line (HeLa) and ovarian cancer cell line (CaOv-3) to screen the antiproliferative activity. The lowest (the best IC50 value) for inhibitory concentration at 50% of cells population (IC50) shows 6.31μg/mL. Treatment of CaOv-3 with *Artocarpus* extract with the lowest IC_{50} value at specific incubation time that is 24 hr, 48hr, and 72hr resulted in inducing apoptosis have been determined by Hoechst stain. Result showed the DNA fragmentation event in the treated cells compared to untreated cells. Treatment of normal cell line, MDCK with the *Artocarpus* extract with the lowest IC_{50} value showed no cytoselective effect in the extract. However the extract inhibits cell growth at high concentration equal to 10μg/mL. These results proposed that *Artocarpus sp.*exhibit antiproliferative activity on HeLa and CaOv-3 which inducing apoptosis in CaOv-3 cells.

PENYARINGAN AKTIVITI ANTIPROLIFERASI OLEH EKSTRAK *Artocarpus* sp. TERHADAP SEL HeLa DAN SEL CaOv-3

ABSTRAK

Artocarpus sp. telah digunakan sebagai ubatan tradisional di Asia Tenggara untuk mengubati ulser, luka, dan cirit-birit. Spesies ini juga dilaporkan berkebolehan untuk merawat keradangan and demam malaria. Dalam kajian ini, tiga spesies *Artocarpus* ekstrak digunakan ke atas dua jenis sel kanser iaitu sel kaser serviks (Hela) dan sel kanser ovari (CaOv-3) untuk penyaringan aktiviti antiproliferasi. Nilai terendah (nilai terbaik IC50) untuk anti-proliferasi bersamaan dengan 6.31μg/mL. Rawatan ke atas CaOv-3 dengan ekstrak *Artocarpus* yang mempunyai nilai IC_{50} yang paling rendah pada masa yang spesifik iaitu 24 jam, 48 jam dan 72 jam menunjukkan kejadian apoptosis terhasil yang telah ditentukan melalui pewarnaan Hoechst. Keputusan ini menunjukkan fragmentasi DNA pada sel yang dirawat berbanding dengan sel yang tidak dirawat. Rawatan terhadap sel normal, MDCK dengan ekstrak *Artocarpus* yang mempunyai nilai IC_{50} yang rendah menunjukkan tiada kesan sitoselektif pada ekstrak. Walaubagaimanapun ekstrak tersebut merencatkan pertumbuhan sel pada kepekatan yang tinggi iaitu 10μg/mL. Keputusan ini menunjukkan bahawa spesies Artocarpus mempunyai aktiviti antiproliferasi pada sel HeLa dan sel CaOv-3, yang turut meransang apoptosis terhadap sel kanser yang diuji.

CHAPTER 1

INTRODUCTION

1.1 Natural products

For thousand years, natural products have important role in treating and preveting disease through out the world. Natural products medicines have some various source material including terrestial plants, terrestial microorganism, marine organism and terrestial vetebrates and invetebrates (Chin *et al.*, 2008).

Nowadays the interest in using alternative therapies and natural product in order to treat diseases has widely growing (Abu *et al.,* 2006). The natural products were the main source of drugs for a long time. The most therapeutic used of natural product is those derived from plant. Terrestial plants have widely been used in treatment of human disease especially higher plants. Historical experiences with plants as therapeutic tools have helped to introduce single chemical entities in modern medicine. Recents studies shows that plants especially those which having ethnopharmacological uses have been the primary source of medicines for early drug discovery (Abu *et al.*, 2006).

1.2 Cancer burden in the world

Cancer had been the most prominent disease in humans and it has been the major factors of developing and continuing discovery of anticancer from natural products. The

use of natural products as an anticancer agents was recognized by United States National Cancer Institute (NCI) in 1950s and become a major contribution to the discovery of new naturally occuring anticancer agents.(Fouche *et al.*, 2008)

Cancer is the one of chronic disease that causes burden for the whole world. World Health Organization (WHO) reported that major causes of death for approximately 6 million men and women every year for the world is cancer in year 2000. It is about 10 million new cases reported every year (Noor, 2008).

On 2002, the numbers of new cases increase about 10.9 million and about 6.7 million of people are reported died because of cancer. WHO state that, the percentage of cancer cases will be increase about 50% for 15 million cases in 2020. The causes of these matter due to high percentage of old people, the incerasing of human life span and the changes of lifestlye that less care about health. Increasing in smoking and practising of unhealthy food intake also can cause increasing in number of cancer every year. Virus infection also can be one of the causes especially in developing country like Hepatitis B (liver cancer) and human papiloma virus, HPV (Noor, 2008).

1.3 Cancer in Malaysia

Based on National Cancer Registration (NCR) in 2003, it is about 21 464 new cases had been reported in Peninsular Malaysia. From the total number of cases, about 9 400 occurance are in men and 12 064 occurance are in women. Basically, the incident of cancer based on aged in men are 134.3 in each 100 000 populations, where as in

women, it is about 154.2 in each 100 000 populations. From the statistical analysis, it shows that breast cancer are having high incident about 3728 cases, followed by lung cancer (men and women) which is about 1758 cases and cervical cancer which is about 1557 cases (Noor, 2008).

Cancer that involve reproductive organ especially in women can give huge impact to the patient. It is because these organs are important as a sexual symbol for a woman. These cancers are not only affecting the physical of the patient but it also can cause trouble for social life of the patients. Patients tend to depress and suffer from mental disturbant.

1.4 Cervical cancer and ovarian cancer

Cervical cancer is a major reproductive organ cancer in our country and also in others developing country. This is the most common cancer occur in women folowed by breast cancer. The major cause of this cancer is human papilloma virus (HPV) infection. Previous study showed that 99.7% of the cervical cancer associated to HPV infection. It is about 100 types of HPV has been identified based on gene arrangement. From these types, it can be divided into two classes that are (Noor, 2008):-

1. Low risk HPV (virus which associate with skin wart)
2. High risk HPV (virus which associates with precancerous and cancer)

Ovarian cancer is one of reproductive organ cancer which is dangerous and difficult to cure. Based on epidemiologic study, three major factors contributed to the

occurrence which are reproductive factor, genetic factor and environment (Noor, 2008). The mechanism of the cancer incident is very complex and there are many other side factors that also can contribute to this cancer.

1.5 Hela cell line and CaOv-3 cell line

In this experiment we used 2 types of cell lines that are HeLa cell lines and CaOv-3 cell lines as the test subject for the plant extract. HeLa cell lines came from cervical cancer cells whereas CaOv-3 came from ovarian cancer cells. We choosed these two types of cell line because these cancers are having the highest mortality rate of all gynecological tumors. Thus, alternative methods for treatment are priority for these cancers.

Hela cells originated from a fatal cervical carcinoma. The human epitelial cells were derived from cervical cancer taken from Henrietta Lacks in 1957. The HeLa genome was created by transferring horizontal gene from human papilloma virus 18 (HPV-18) to human cervical cells. It is different from parent genome in various ways including its chromosome number. HeLa cells are adherent cells which maintain contact inhibition *in-vitro*. It is an example of immortalized cell line that widely used for *in-vitro* studies.

CaOv-3 cell line was derived from ovarian cancer cells (Hurst and Hooks, 2009). This is another type of cancer cell line that widely use for in-vitro studies. Usually people tend to use this cell line in order to identify of appropiate tumor selective antigen

(Ag) for development of succesful ovarian tumor specific imunotherapy (Hur *et al.*, 2007).

1.6 Normal cell (MDCK cells)

MDCK cells were derived from a normal kidney of female adult cocker spania in September 1958. It was discovered by S.H. Madin and N.B. Darby which cause the cells known as Madin-Darby Canine Kidney cells or MDCK. These cells can be used at various passage levels in viral study (Youil *et al.*, 2004).

In this study, MDCK cells had been used as control. This is to ensure that the extract or the compound did not give negative effect on normal cells and it is very good for treating cancer cells (Mena-Rejon *et al.*, 2009).

1.7 *Artocarpus* sp.

In this experiment we had used plant from genus of *Artocarpus* from family of Moracea. The name *Artocarpus* was derived from Greek words *artos* = bread and *karpos* = fruit. This name was appointed by Johann Reinhold Forster and J. George Adam Forster. This genus of *Artocarpus* have characteristic like monocieous trees which have spiral leaves or distichious. These plants can produce milky sap. Male flower are surrounded with peltate to clavate interfloral bract whereas female flowers are at least partially adnate to each other or to interfloral bract. The flowers and bract are laterally fused to form syncarp.

There is about 50 species at tropical and subtropical Asia, Pacific Island and 14 species in China. Some species are important for their fruit and timber. Most of the species are native to Asia and there are producing starchy fruits that are frequent staples (Janick and Paull, 2008).

Taxonomy	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Hamamelidae
Order	Urticales
Family	Moracea
Genus	Artocarpus

Table 1.1 Taxonomy of *Artocarpus* sp.

In this experiment we used three species of Artocarpus that are *A. anisophyllus*, *A. lanceifolius*, and *A. maingayi*. These plants lead us to investigate cytotoxic activity of the compoud that can act as anticancer in order to produce new natural agent for cancer treatment.

A.anisophyllus is an evergreen tree that can be up to 25m tall. It can be found in Borneo, Kalimantan and Southern Sumatra. It produce round shape of fruits which having yellow to brown color and the size of the fruits within 7- 10 cm. The fruits flesh is orange in color and have sweet taste. These species also called as klidang, mentawa,

entawa, keledang babi, and pupuan. It is cultivated for timber in its native range (Janick and Paull, 2008).

Figure 1.1 *A. anisophyllus*

(Source: http://commons.wikimedia.org/wiki/File:Arto_aniso_T_070203_mncg.JPG and http://pick5.pick.uga.edu/mp/20p?see=I_NZ3)

For the second species that are *A. lanceifolius*, it is commonly evergreen tree that grown up to 36m tall. It usually found in lowland and also hill forest that altituted around 600m in Thailand, peninsular Malaysia, Sumatra, Bangka, and north eastern Borneo. The fruits can be up to 7cm in diameter. These trees are source of timber and dye. In Malaysia, Sumatera and Kalimantan, this species is known as Keledang whereas in Thailand it is

known as khanun-pa. It is also known as simar baka for Batak, Sumatra and bangsal for Dayak, Kalimantan (Janick and Paull, 2008).

For the third species is *A. maingayii*. This species is medium size or large evergreen tree up to 40m tall. It is usually found in lowland evergreen forest up to 150m altitude. It mostly distributed in Sumatra. This species commonly used as hard wood for house and boat bulding. (Hakim *et al.*, 2006)

1.8 Research Objectives

The objectives of this study are:-

1. To screen the anti-proliferative activity of *Artocarpus* sp. extract on Hela and CaOv-3 cell lines.

2. To determine DNA fragmentation event in the cell line treated with the most active extract of *Artocarpus* sp.

CHAPTER 2

LITERATURE REVIEW

2.1 *Artocarpus* sp.

Artocarpus species has been an important source of edible fruit such as *A. heterophyllus* (Jack fruit), *A. chempeden* (Chempedak) and *A. altilis* (Bread fruit). Some *Artocarpus* have become source of timber. *Artocarpus* species also used in traditional folk medicine in Southeast Asia for treating ulcer, abscess, and diarrhea. This species has been reported to be used for treatment of inflammation, and malarial fever (Arung *et al.*).

Previous study reported that, it is about 25 isoprenyleatedflavanoid were presented and many of the phenolic constituents isolated from *Artocarpus* sp. investigates were significant bioactive compound (Hakim *et al.*, 2006).

Artocarpus sp. contain abundant source of phenolic constituents. These constituents can be classified into isoprenylflavanoid, stilbenoid and 2-arylbenzofuran derivatives. The flavanoid can be characterized by its unique features like the chemical structures containing isoprene substituent and certain pattern of oxygenation of the flavone skeleton (Hakim *et al.*, 2006).

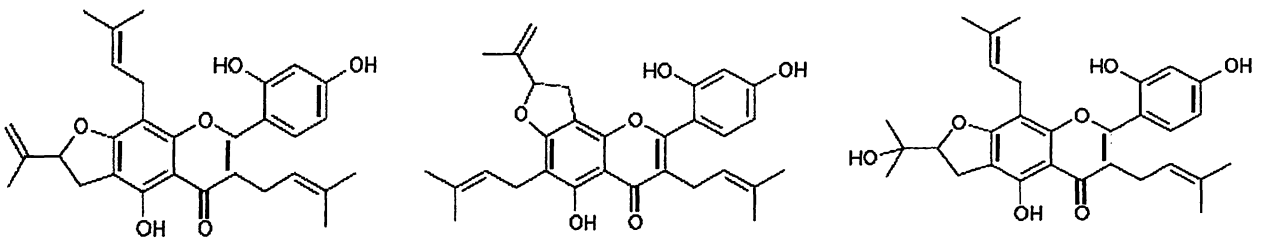
This isoprenylatedflavanoid also showed potent cytotoxic activity against various cell lines like murine leukemia P388, nasopharynx carcinoma (KB), mouse L-210 and

colon 38. It is also have antibacterial activity against cryogenic bacteria, antiplatelet activity, and can cause inhibiton of arachidonate 5-lipoxygenase (Arung *et al.*).

2.1.1 *A. lanceifolius*

Previous study reported that heartwood and tree barks of *A. lanceifolius* yield a number of compound that have certain biosynthetic capacity of *Artocarpus* with regards to 3-isoprenylated flavones and their transformation product (Syah *et al.*, 2001).

Based on this study, several prenylflavone derivatives basically based on 3, 6, 8 – triprenylated flavone possessing a 2', 4'-dioxygenated B-ring. Other than that, artoindonesianins G-I (Figure 2.1), artelastofuran (4), and artelasticin (5) (Figure 2.2) were isolated from benzene and chloroform-soluble fraction of methanol extract from the heartwood of *A. lanceifolius*. These compounds show strong cytotoxicity against cancer cell line (Syah *et al.*, 2001).



1 2 3

Figure 2.1 Structure of artoindonesianin G-1