

**ANTIPROLIFERATIVE ACTIVITY OF *Brucea javanica* FRUITS  
EXTRACT TOWARDS HeLa CANCER CELL LINES**

**by**

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degree of Bachelor of Health Sciences (Biomedicine)  
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**CERTIFICATE**

This is to certify that the dissertation entitle  
**Antiproliferative Activity of *Brucea javanica* Fruits Extract**  
**Towards HeLa Cancer Cell Lines**

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# The List of Abbreviations, Symbols or Nomenclature

## The List of Abbreviations

ACS	American Cancer Society
ATCC	American Type Culture Collection
BJF	<i>Brucea javanica</i> fruit
CO <sub>2</sub>	Carbon dioxide
CR	Crude Incidence Rate
DMEM	Dulbecco's Modified Minimum Essential Medium
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
HCl	Hydrochloric acid
IC <sub>50</sub>	Median concentration that causes 50% inhibition (inhibitory concentration)
MOS	Malaysian Oncological Society
n.d	Not dated
NaCl	Sodium chloride
NaHCO <sub>3</sub>	Sodium bicarbonate

<b>NCI</b>	<b>National Cancer Institute</b>
<b>PBS</b>	<b>Phosphate Buffer Saline</b>
<b>Pen/ strep</b>	<b>Penicillin Streptomycin</b>
<b>Pet-Ether</b>	<b>Petroleum- Ether</b>
<b>PBUH</b>	<b>Peach be Upon Him</b>
<b>S.A.W</b>	<b>Salawallahialaihiwassalam</b>
<b>SEM</b>	<b>Standard Error Mean</b>

## The list of Symbols

%	Percentage
<	Less than
>	More than
±	Plus Minus
≤	Less or equal
≥	More or equal
μg	Micrograms
μg/ml	Microgram per milliliter
cm <sup>3</sup>	Square centimeter
g	grams
Hz	Hertz
M	Mole
mg	milligrams
mg/ml	Milligram per milliliter
mm	millimeter
OD	Optical density
rpm	Round per minutes
v/v	Volume per volume
μl	Micro liter
nm	Nanometer

## ABSTRACT

Fruits of *Brucea javanica* (*B. javanica*) has been used as a folk remedy in Chinese medicine, and it has been shown to exhibit antiamebic, antimalarial, antiplasmodial, antileukaemic and anticancer. In this study, *B. javanica* fruit (BJF) were extracted using petroleum ether, methanol and distilled water as solvent. All three crude extract were treated on human cervical adenoma cell line, HeLa and human breast cancer cell line, MCF-7. All the extract showed IC<sub>50</sub> value less than 100µg/ml and methanol crude extract on HeLa cells were given the best IC<sub>50</sub> value with 2.8 µg/ml. Thus, HeLa cells were selected to undergo microscopic analysis examination using methanol crude extract of BJF. The analysis of cellular morphology and nuclei morphology on treated HeLa cells at 24, 48 and 72 h were done by Hematoxylin and Eosin (H&E) staining and Hoechst 33258 staining, respectively. The typical apoptosis characteristics were observed in H&E stain. The apoptotic indexes of BJF-treated cells were revealed almost similar degree of apoptosis at time dependent manner compare with positive control, tamoxifen. These results proposed that BJF exhibits antiproliferative effect on HeLa cells via inducing-apoptosis. Therefore, it may be a potential candidate in field of anticancer drug discovery.

## ABSTRAK

Buah *Brucea javanica* (*B. javanica*) digunakan dalam perubatan China sebagai antiamoebik, antimalaria, antiplasmodial, antileukemia dan antikanser. Ekstrak kasar buah *B. javanica* menggunakan petroleum eter, metanol dan air sebagai pelarut telah dijalankan ke atas kanser adenoma servik (HeLa) dan sel kanser payudara (MCF-7). Semua ekstrak memberikan nilai  $IC_{50}$  yang kurang daripada  $100\mu\text{g/ml}$  dan ekstrak methanol telah memberikan hasil yang terbaik dengan nilai  $IC_{50}$   $2.8\ \mu\text{g/ml}$  terhadap titisan sel kanser adenoma servik, HeLa. Oleh yang demikian, sel-sel HeLa telah dipilih untuk menjalankan analisis morfologi secara mikroskopik dengan menggunakan ekstrak kasar methanol. Kajian morfologi sel dan nukleus dijalankan selepas merawat sel-sel HeLa pada masa 24 j, 48 j dan 72 j dengan menggunakan kaedah pewarnaan Hematoxilin dan Eosin (H&E) dan juga pewarnaan Hoechst 33258. Rupa bentuk apoptosis yang tipikal dilihat pada sel-sel HeLa. Indeks apoptosis oleh sel-sel HeLa yang dirawat dengan BJF memberikan keputusan yang hampir sama dengan darjah apoptosis sel-sel kawalan positif yang dirawat dengan tamoxifen apabila berkadaran dengan masa. Hasil penemuan ini mencadangkan BJF mempamerkan kesan antiproliferasi ke atas sel-sel HeLa melalui induksi apoptosis. Oleh yang demikian, ia mungkin berpotensi sebagai calon kepada penemuan ubat antikanser yang baru.



## **1.0 Introduction**

Malaysia's rich and diverse tropical rainforests have been recognized internationally as a depository of megadiversity of both flora and fauna and act as a large storehouse of untapped genetic resources (Lee *et al.*, 2004). At present, forests of Malaysia is estimated contain some 14 500 species of flowering plants (Anon, 2001). However, only 1200 species is utilized in traditional medicine (Wiat *et al.*, 2000). Thus, the floras in Malay Peninsula remain as vast untapped reservoirs for exploration and screening of plants for pharmacologically active molecules (Wiat *et al.*, 2000).

The World Health Organization estimate that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care (Farnsworth *et al.*, 1985). The remaining 20% of individuals living in the first world use, in more than 25% of cases, pharmaceuticals which have been directly derived from plant products (Farnsworth, 1984 and Cox, 1994). Approximately 75% natural product substances were discovered as a direct result of chemical studies focused on the isolation of active substances from plants used in traditional medicine (Cragg *et al.*, 2001).

The American Cancer Society (ACS) estimates that about 1.4 million new cases of cancer are expected in 2007, and about 560,000 people will die of the disease (ACS Cancer Statistics 2007). Cancer refers to any one of a large number of diseases characterized by the development of abnormal cells that uncheck growth that progresses toward limitless expansion and have the ability to infiltrate and destroy normal body tissue. Cancer can

spread throughout the body. Being diagnosed with cancer can be frightening. It sounds going to death. Anywhere part of the body cancer can be originated. It is due to normal cell in our body has the potential to become cancer cell (Weinberg, 1996).

Cancer is an increasing health trouble in Malaysia. Cancer has been reported as the fourth leading cause of death in Malaysia (Lim *et al.*, 2002). While the idea of a National Cancer Registry was first reported in the Star on 7 April 1978, the First Report of the National Cancer Registry was only realised on 4 July 2003 (Lim *et al.*, 2002). For the first time, the real cancer burden in Malaysia was confidently estimated. A total of 26,098 patients were diagnosed with cancer among all residents in Peninsular Malaysia in 2002 (Lim *et al.*, 2002). The corresponding figures for Sabah and Sarawak were 1,748 and 2,002, respectively (Lim *et al.*, 2002).

The treatment of cancer relies on the type of cancer as well as the stages of that disease. Normally the goal of treatment is to complete removal of the cancer without damage to the rest of the body. Treatment of cancer is a multidisciplinary effort (Bela *et al.*, 1995). The modalities of treatment include surgery, radiotherapy, chemotherapy, hormonal therapy, immune therapy and symptomatic as well as supportive therapy. However, the propensity of cancers to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limits surgery effectiveness. The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Radiation can also cause damage to normal tissue (Eliot *et al.*, 2000).

The need to find a safe and highly effective cure for neoplastic disease remain a major challenge for modern science (Alexandrova, 2000). Throughout medical history, plant products have been shown to be valuable sources of novel anti-cancer drugs (Hartwall, 1982 and Huang, 1999). When new plant-derived therapeutics based on indigenous knowledge are being explored, it is important that the companies return benefits to the native population and the local governments from which the research material was obtained (King *et al.*, 1996). Many tropical plants have interesting biological activities with potential therapeutic applications.

*B. javanica* is one of the plants that can be found in Malaysia which is well known as promising cancer chemopreventive. Discovery of potential activity of this plant merely undeveloped was made me interest to explore it. Recent data suggested that a lot of research had been conducted using this plant. However, lack of resources in early 1980's had resulted it being dropped from consideration.

## **1.1 Introduction of *B. javanica***

*B. javanica* is a shrub or small tree with 1 to 3 m high and normally the younger parts softly pubescent. It leaves compound is paripinnate with leaflets 5 to 11, oval-lanceolate, 5–10 cm long by 2 to 4 cm wide; apex acuminate, base broadly cuneate and often somewhat oblique; margin serrate; both surfaces densely pubescent, especially the underside. Fruit and drupe ovoid, black when ripe. Seeds, compressed, rugose, blackish brown (WHO, 1990).

This plant commonly found in Monsoon Tropica's forest. Found in Malaysia, India through to southern China and down into northern Australia. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It requires moist soil. It grows well under humid and seasonal weather conditions to an altitude of 900m (Samy *et al.*, 2005).

### 1.1.1 Scientific Classification for *B. javanica*

Kingdom : Plantae  
Division : Magnoliophyta  
Class : Magnoliopsida  
Order : Sapindales  
Family : Simaroubaceae  
Genus : *Brucea*  
Species : *javanica*



Figure 1 *B. javanica* plant (www.melur.com, 2007)

### 1.1.2 Fruits of *B. javanica*

Fructus Bruceae consists of the dried ripe fruits of *B. javanica* (L.) Merr. (Simaroubaceae). *Brucea amarissima* Desv. ex Gomes, *B. sumatrana* Roxb., *Gonus amarissimus* Lour., and *Lussa amarissima* O. Ktze are the synonyms of Fructus Brucea (WHO, 1990). Selected vernacular names including Biji makassar, bulah makassar, Java brucea, k'u-shen-tzu, kho sam, ko-sam, kusheng-tzu, nha dàm tùr, raat cha dat, raat dat,

ratchadat, sàu dau rùng, xoan rùng, ya tan tzu, ya-dan-zi, yadānzi ,Ya Dan Zi, *Rhus chinensis*, Chinese gall, Java brucea , Lada Pahit or Macassar kernel tree (Mill, 1997 & Steve 2004). The kernel or the seed that pulp removed also refer as fruit (WHO, 1990).

General appearance of the fruit is ovoid, 6 to 10 mm long by 4 to 7 mm in diameter. Externally black or brown, with raised reticulate wrinkles, the lumen irregularly polygonal, obviously ribbed at both sides. Apex acuminate, base having a dented fruit stalk scar, shell hard and brittle. Seeds ovoid around 5 to 6 mm long by 3 to 5 mm in diameter, externally yellowish white, reticulate; testa thin, cotyledons milky white and oily.

Organoleptic properties for *B. javanica* fruit are odour slight and very bitter taste. Microscopic characteristics show off brown pulverized pericarp. Epidermal cell polygonal with cellular contents is brown; parenchymatous cells also polygonal containing cluster of calcium oxalate prisma up to 30 μm in diameter. Stone cells is 14-38 μm in diameter is sub rounded or polygonal in shape (WHO, 1999).

Purity testing in fruit's of *B. javanica* product on *Salmonella* spp. should be negative. For decoction preparation: aerobic bacteria not more than  $10^7$ /g; fungi-not more than  $10^5$ /g; *Escherichia coli*-not more than  $10^2$ /g. meanwhile the capsule preparation for internal use for example: aerobic bacteria- not more than  $10^5$ /g; fungi-not more than  $10^4$ /g; enterobacteria and certain Gram-negative bacteria-not more than  $10^3$ /g; *Escherichia coli*- 0/ g. (WHO, 1999).

Although a lot of claim, it can heal many diseases but WHO Monographs on *B. javanica* fruits stated that it have been used clinically in the treatment of amoebic dysentery. However, it should not be administered to children, pregnant women or nursing mother since teratogenic and non-teratogenic effect have not been establish yet. No information available about general precautions or precautions concerning carcinogenesis, mutagenesis or impairment of fertility; drug interactions; or drug and laboratory test interactions.

In term of posology, daily dose to treat amobiasis is 4-16 g as a decoction or powder in three divided doses for 3-7 days (WHO, 1990) meanwhile for treat malaria required 3-6 g in three divided doses after meals for 4 or 5 days (WHO, 1990). For adverse reactions had been noticed of some cases of anaphylaxis after external application of the fruits of *B. javanica* (Zheng *et al.*, 1986).



Figure 2 Fresh compound leaves of *B. javanica* with aggregates fruits.



Figure 3 General appearance of the dried fruit of *B. javanica* in bunch.

### 1.1.3 Ethnopharmacological

Ethnopharmacological data can be obtained by consulting traditional healer and by accumulating information on the popular medicinal use of plants, but also from literature on folk medicine (Duke, 1985., Yeung, 1985 and Chopra, 1986). Ethnopharmacological data becomes the important source of new drugs. The leaves and the roots of *B. javanica* are depurative. They stimulate blood circulation. A decoction is used in the treatment of haemoptysis, inflammations, laryngitis, snakebite, stomach-ache and traumatic fractures (Duke, 1985., Yeung, 1985 and Chopra, 1986). The stem bark is astringent and anthelmintic. The fruit is used in the treatment of colic (Duke, 1985., Yeung, 1985 and Chopra, 1986). The seed is used in the treatment of coughs, dysentery, fever, jaundice, malaria and rheumatism. The root bark is cholagogue (Duke, 1985., Yeung, 1985 and

Chopra, 1986).

Galls on the plant are used internally for their astringent and styptic properties. They are a frequent ingredient in polyherbal prescriptions for diabetes mellitus. An excrescence produced on the leaf by an insect *Melaphis chinensis* or *M. paitan* is antiseptic, astringent and haemostatic. It is used in the treatment of persistent cough with blood, chronic diarrhoea, spontaneous sweating, night sweats, bloody stool, uorrhoea and bloody sputum. It is used applied externally to burns, bleeding due to traumatic injuries, haemorrhoids and ulcers in the mouth (Duke, 1985., Yeung, 1985 and Chopra, 1986).

#### **1.1.4 Early Phytochemical studies**

Plants from Simaroubaceae are known to contain compounds with highly oxygenated triterpenes and bitter taste called as quassinoids (Khosa *et al.*, 1985). Initially the compounds of such chemical nature were known by the term “quassin” after the physician “Quassi” who used the bark of plants from this family for the treatment of fever. Studies on quassinoids have shown their promising role as therapeutic agents as an antitumor, antiviral (Polonsky *et al.*, 1985 and Apers *et al.*, 2002), anti-inflammatory, antiamoebic (Duriez, 1962) antimalarial (Chulabbon *et al.*, 1994 and Philipson *et al.*, 1997), insecticidal, antitubercular (Rahman *et al.*, 1997), anticancer (Varieota *et al.*, 1998), amoebicidal (Polonsky *et al.*, 1985), antiulcer (Toma *et al.*, 2002), herbicidal and antifeedent, etc.