



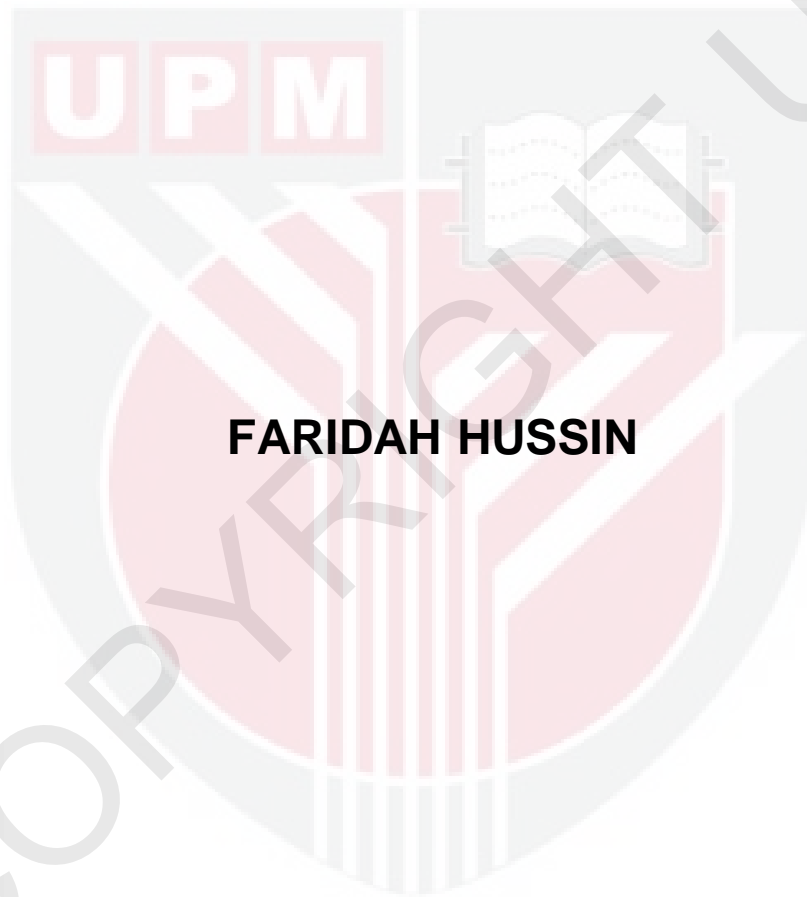
**UNIVERSITI PUTRA MALAYSIA**

**POTENTIAL CHEMOPREVENTION OF PRIMARY LIVER CANCER USING  
OPTIMIZED FORMULATION OF STROBILANTHUS CRISPUS (L.) BLUME  
AND CENTELLA ASIATICA L. JUICES**

**FARIDAH HUSSIN**

**IB 2011 21**

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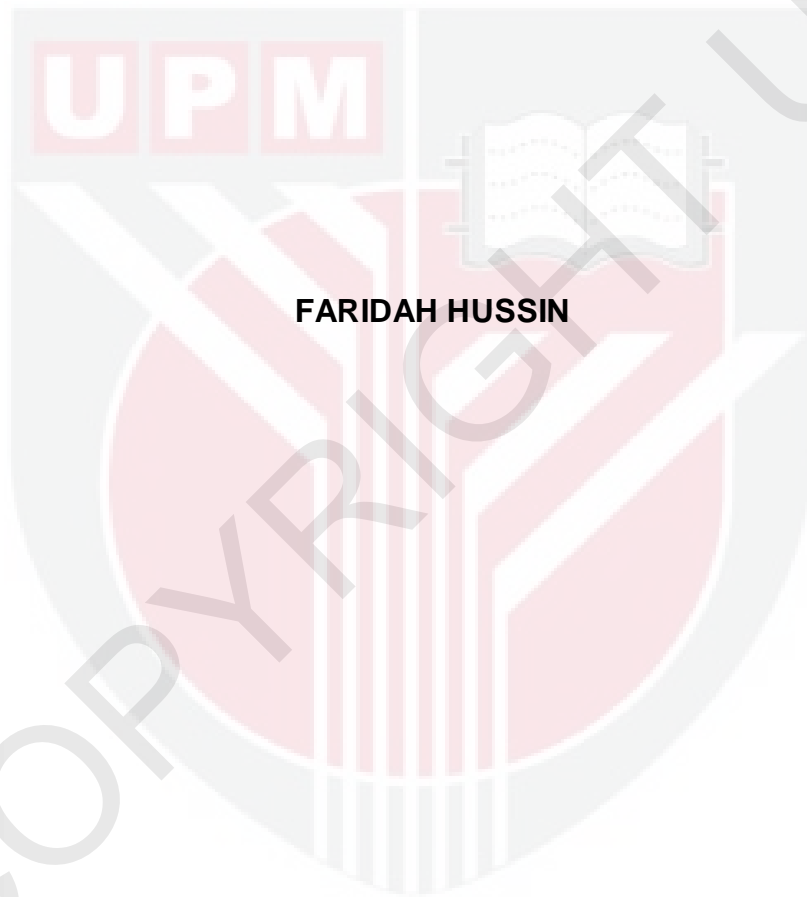


**FARIDAH HUSSIN**

**DOCTOR OF PHILOSOPHY  
UNIVERSITI PUTRA MALAYSIA**

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**FARIDAH HUSSIN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia in Fulfilment of the Requirement of the Degree of Doctor of  
Philosophy**

**2011**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

**POTENTIAL CHEMOPREVENTION OF PRIMARY LIVER CANCER  
USING OPTIMIZED FORMULATION OF STROBILANTHUS CRISPUS  
(L.) BLUME AND CENTELLA ASIATICA L. JUICES**

By

**FARIDAH HUSSIN**

**August 2011**

**Chairman: Professor Asmah Rahmat, PhD**

**Faculty: Medicine and Health Sciences**

This study was conducted to evaluate the chemoprevention potential of *Strobilanthes crispus* and *Centella asiatica* juices on liver cancer cell line (HepG2). The first and second parts of the study were on the optimization of juices formulation and processing condition for the development of high quality juices using response surface methodology. The combined effect of two independent variables for formulation optimization i.e. herbal puree (14-24%) and honey (14-40%), and two independent variables for processing condition i.e. temperature (60-80<sup>0</sup> C) and time (8-30 min) were studied. Response surface plots showed increase in herbal and honey concentration, temperature and time of processing reduced the response variables significantly ( $p < 0.05$ ). The optimum product formulation for *Centella asiatica* and *Strobilanthes crispus* juices singly were 14% of both herb and honey concentration. The optimum parameter for processing of *Centella asiatica* juice was at 70°C for 8 min whereas the optimum parameter for processing of *Strobilanthes crispus* juice was at 69°C for 8

min. The third part of the study focused on the cytotoxic activities of juices at different concentration (0.001, 0.01, 0.1, 1 and 10%) and incubation time (24, 48, 72 hours). MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide) assay was used to examine the cytotoxic effect of juices on Chang cell line as control and HepG2 cells. Increasing doses of both juices up to 10% had no cytotoxic effect against Chang cell line as indicated by absence of IC<sub>50</sub> values when the cell lines were treated for up to 72 hours incubation time. However, *Strobilanthes crispus* juice and *Centella asiatica* juice inhibited the proliferation of HepG2 cancer cell lines in a concentration-dependent manner as well as exposure time. The cytotoxicity started at a concentration as low as 0.1% for both juices. At 72 hours incubation time, *Strobilanthes crispus* and *Centella asiatica* juice showed cytotoxicity effects with an IC<sub>50</sub> of approximately 0.4 (4 mg/L) and 0.1% (1 mg/L) respectively. The fourth part of this study was to discuss on the apoptotic morphology of HepG2 cancer cells treated with both juices by quantitatively measuring the apoptotic cell percentage using flow cytometry and comet assay. Both juices induced apoptosis in a dose-dependent manner after being exposed to HepG2 cell lines for 72hr. At more than 0.1% concentration of juices, both *Centella asiatica* and *Strobilanthes crispus* showed higher percentage of apoptotic cell death (sub-G1 population) than the control HepG2 cells. Comet assay also showed that all cells exhibited a dose-dependent increase in DNA damage compared to controls. A significant induction of DNA damage was seen at concentrations of above 0.1% for both juices. Finally the last

part of the study was to observe the changes in the c-myc, c-fos and c-erbB2 oncogenes expression induced by the *Centella asiatica* and *Strobilanthes crispus* juices. Results revealed that *Centella asiatica* juice reduced level of c-myc but increased level of c-fos and c-erbB2 expression on HepG2 cells. Contrary, *Strobilanthes crispus* juices showed increased level of c-myc with reduced level of c-fos and c-erbB2 expression on HepG2 cells. In conclusion, *Centella asiatica* and *Strobilanthes crispus* juices have potential as chemopreventive agents in liver cancer cell.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**POTENSI PENCEGAHAN KIMO TITISAN KANSER HATI PRIMER  
MENGUNAKAN FORMULASI OPTIMA JUS PECAH BELING  
(*STROBILANTHES CRISPUS (L.) BLUME*) DAN PEGAGA  
(*CENTELLA ASIATICA L.*)**

Oleh

**FARIDAH HUSSIN**

Ogos 2011

**Pengerusi: Professor Asmah Rahmat, PhD**

**Fakulti: Perubatan dan Sains Kesihatan**

Kajian ini dijalankan untuk menilai potensi pencegahan-kimo jus pecah beling (*Strobilanthes crispus*) dan pegaga (*Centella asiatica*) keatas sel kanser hati primer. Bahagian pertama dan kedua kajian adalah untuk mengoptimum formulasi dan parameter pemprosesan bagi menghasilkan jus yang berkualiti tinggi dengan menggunakan kaedah permukaan gerak balas (response surface methodology). Kajian kesan kombinasi 2 pemboleh ubah bebas untuk formulasi iaitu puri herba (14-24%) dan madu (14-40%), dan 2 pemboleh ubah bebas untuk kondisi pemprosesan iaitu suhu (60-80 °C) dan masa (8-30 min) telah dijalankan. Plot permukaan gerak balas menunjukkan pertambahan kepekatan puri herba dan madu, dan kenaikan suhu dan masa pemprosesan telah menurunkan pemboleh ubah gerak balas secara signifikan ( $p < 0.05$ ). Formulasi optima jus pecah beling (*Strobilanthes crispus*) dan pegaga (*Centella asiatica*) masing-masing adalah 14% untuk kedua-dua puri and madu. Parameter pemprosesan optima jus pegaga (*Centella asiatica*) adalah

pada suhu 70°C selama 8 minit manakala jus pecah beling (*Strobilanthes crispus*) pada suhu 69°C selama 8 minit. Bahagian ketiga kajian fokus kepada kajian aktiviti sitotoksiti jus pada berbeza kepekatan (0.001, 0.01, 0.1, 1 and 10%) dan masa inkubasi (24, 48, 72 jam). Asai MTT (3-(4, 5-dimethylthiazole-2yl)-2, 5-diphenyl tetrazolium bromide) telah digunakan untuk mengkaji kesan sitotoksik jus terhadap sel hati primer normal (Chang) sebagai kawalan dan sel barah hati primer (HepG2). Pertambahan pada dos kedua-dua jus sehingga 10% tiada menunjukkan kesan sitotoksik terhadap sel hati primer normal apabila tiada nilai Perencatan Kepekatan ( $IC_{50}$ ) semasa sel dirawat sehingga 72 jam. Walaubagaimanapun, jus pecah beling (*Strobilanthes crispus*) dan pegaga (*Centella asiatica*) merencat pembiakan sel barah hati primer bergantung kepada kepekatan dan masa rawatan. Sitotoksiti bermula pada kepekatan serendah 0.1% untuk kedua-dua jenis jus. Jus pecah beling (*Strobilanthes crispus*) dan pegaga (*Centella asiatica*) menunjukkan kesan sitotoksiti masing-masing dengan Perencatan Kepekatan ( $IC_{50}$ ) pada 0.4% (4 mg/L) and 0.1% (1 mg/L). Bahagian ke empat kajian ini membincangkan tentang apoptotik sel barah hati primer dirawat dengan kedua-dua jus dengan mengukur secara kuantitatif peratus sel menggunakan arusan sitometri dan asai komet. Kedua-dua jus menggalakkan apoptotik pada perkadaran terus dengan kepekatan apabila didedahkan kepada sel barah hati primer selama 72 jam. Pada kepekatan lebih daripada 0.1%, kedua-dua jus *Centella asiatica* dan *Strobilanthes crispus* menunjukkan lebih tinggi peratus sel mati apoptotik



(populasi sub-G1) berbanding sel barah hati primer kawalan. Asai komet juga menunjukkan kesemua sel mempamerkan pertambahan pada perkadaran terus kepekatan kerosakan DNA berbanding sel kawalan. Penambahan kerosakan DNA yang signifikan boleh diperhatikan pada kepekatan lebih daripada 0.1% kedua-dua jus. Bahagian terakhir kajian memerhatikan perubahan ekspresi onkogen c-myc, c-fos dan c-erbB2 sel barah hati primer dirawat dengan jus *Centella asiatica* dan *Strobilanthes crispus*. Keputusan menunjukkan jus *Centella asiatica* menindas onkogen c-myc tetapi meningkatkan ekspresi onkogen c-fos and c-erbB2 didalam sel barah hati primer. Berbeza pula dengan jus *Strobilanthes crispus* dimana ia meningkatkan ekspresi onkogen c-myc dengan menindas ekspresi onkogen c-fos dan c-erbB2. Sebagai rumusan, jus *Centella asiatica* dan *Strobilanthes crispus* berpotensi sebagai agen pencegahan kemo pada sel barah hati primer.

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I certify that a Thesis Examination Committee has met on 1<sup>st</sup> August 2011 to conduct the final examination of Faridah Hussin on her thesis entitled "Potential Chemoprevention of Primary Liver Cancer using Optimized Formulation of *Strobilanthus crispus* (L.) Blume and *Centella asiatica* L. Juices " 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 degree of Doctoral of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Associate Professor Dr. Sabariah bt Abdul Rahman**

UPM-MAKNA Cancer Research Laboratory  
Institute Bioscience  
Universiti Putra Malaysia  
(Chairman)

**Professor Dr. Rozita bt Rosli**

Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Associate Professor Dr. Chong Pei Pei**

Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Professor Dr. Ranjana Prasad Bird**

Vice President Research  
Sunset Anevue Windsor  
N9b 3p4 Ontario  
Canada  
(External Examiner)

---

**SEOW HENG FONG, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 22 November 2011

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

**Asmah Rahmat, PhD**

Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Fauziah Othman, PhD**

Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Abdah Mohd Akim, PhD**

Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

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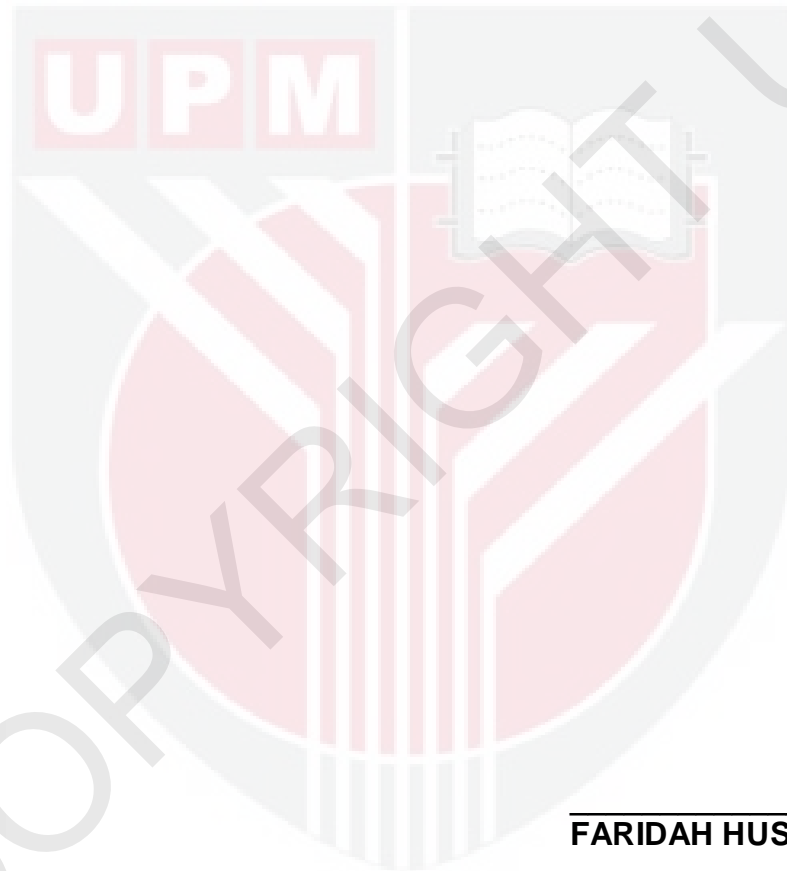
**BUJANG BIN KIM HUAT, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



**FARIDAH HUSSIN**

Date: 1 August 2011

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