UNIVERSITI PUTRA MALAYSIA

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

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IB 2011 21
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DOCTOR OF PHILOSOPHY
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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 STROBLANTHUS CRISPUS (L.) BLUME AND CENTELLA ASIATICA L. JUICES

By

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August 2011

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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°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
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$_{50}$ 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$_{50}$ 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 MENGGUNAKAN FORMULASI OPTIMA JUS PECAH BELING (STROBLANTHES CRISPUS (L.) BLUME) DAN PEGAGA (CENTELLA ASIATICA L.)

Oleh

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Pengerusi: Professor Asmah Rahmat, PhD
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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 sitotoksisiti 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 sitotoksis 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 sitotoksis 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. Sitotoksisiti bermula pada kepekatan serendah 0.1% untuk kedua-dua jenis jus. Jus pecah beling (Strobilanthes crispus) dan pegaga (Centella asiatica) menunjukkan kesan sitotoksisiti 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.
ACKNOWLEDGEMENTS

‘In the name Allah, The Most Gracious, The Most Merciful’

First and foremost, I thank Allah for his blessings and guidance in my life as human and for what I have achieved so far.

I would like to express my sincere appreciation and gratitude to Professor Dr Asmah Rahmat (Supervisor), Professor Dr Fauziah Othman (Co-supervisor) and Dr Abdah Md Akim (Co-supervisor) for their guidance, invaluable advice and support, and understanding throughout the study. The endless interest on my research project, invaluable suggestions and remarks from the supervisory committees help me to complete this study successfully.

My deep gratitude and thanks to all my friends and staffs especially Dr. Zolkafl Eshak, Ms. Normah Ahmad, Ms. Rahimah Mohd Zaki, Mr. Mohd Zain Din, Ms. Nurul Izzah and those who has directly and indirectly involved during the implementation of the experiment and preparation of the thesis. My special love and gratitude go to all my family members especially my mother, Halipah Midon and sisters: Umi Noor Saa’diah and Umi Noor Saa’dah for their continuous moral support and pray. Finally, my love and appreciation to my wonderful husband: Zaharudin, and sons: Muhammad ‘Atif, Muhammad ‘Afif and Ahmad Danish Arman who have always been an anchor and supporter in my life.
I certify that a Thesis Examination Committee has met on 1st 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.

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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
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>ix</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF ANNOTATIONS AND ABBREVIATIONS</td>
<td>xx</td>
</tr>
</tbody>
</table>

# CHAPTER

1 **INTRODUCTION**

2 **LITERATURE REVIEW**

2.1 Primary Liver cancer (Hepatocellular carcinoma) and its causes

2.2 Chemoprevention of cancers using plant sources

2.3 Role of herbal products in chemoprevention

2.3.1 *Centella asiatica*

2.3.2 *Strobilanthes crispus*

2.4 Processing of herbal juices using response surface Methodology (RSM)

2.5 Response surface methodology

2.6 Cell death and Apoptosis in cancer

2.7 Flow cytometry

2.8 Comet assay

2.9 Critical DNA Targets: Protooncogenes and Tumor Suppressor

3 **MATERIALS AND METHODS**

3.1 Raw materials

3.2 Preparation of herbal juices

3.3 Experimental design and statistical analysis

3.4 Determination of product properties for formulation optimum

3.4.1 Physical characteristics

3.4.2 Sensory evaluations
3.5 Determination of product properties for processing condition optimization
   3.5.1 Determination of chlorophyll content
   3.5.2 Determination of vitamin C
   3.5.3 DPPH free radical scavenging activity
   3.5.4 Microbiological test
3.6 Cytotoxicity study and antiproliferative study
   3.6.1 Preparation of herbal juices
   3.6.2 Cell culturing
   3.6.3 MTT assay
3.7 Apoptotic study
   3.7.1 Flow cytometry analysis using RNaseA/Propidium iodide
   3.7.2 Comet assay
   3.7.3 Cell treatments
   3.7.4 Comet assay analysis
   3.7.5 Comet capture and analysis
   3.7.6 Scoring
3.8 Mechanism of gene expression using molecular approach
   3.8.1 Treatment of cells
   3.8.2 RNA purification
   3.8.3 Precipitation of Total RNA
   3.8.4 Removal of contaminating DNA from RNA preparations
   3.8.5 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)
   3.8.6 Agarose Gel Electrophoresis
3.9 Statistical analyses

4 RESULTS
4.1 Optimization of product formulation of Centella asiatica and Strobilanthes crispus juices using response surface methodology (RSM)
4.2 Model description from RSM
4.3 Physical characteristics of Centella asiatica and Strobilanthes crispus juices
4.4 Sensory attributes of Centella asiatica and Strobilanthes crispus juices
4.5 Optimization of formulation establishment
4.6 Optimization of processing parameters for the development high quality Centella asiatica and Strobilanthes crispus juices using response surface methodology (RSM)
4.7 Model fitting from RSM
4.8 Effect of temperature and time on color of Centella asiatica and Strobilanthes crispus juices
4.9 Effect of temperature and time on vitamin C content of
Centella asiatica and Strobilanthes crispus juices

4.10 Effect of temperature and time on chlorophyll content of
Centella asiatica and Strobilanthes crispus juices

4.11 Effect of temperature and time on total antioxidant of
Centella asiatica and Strobilanthes crispus juices

4.12 Optimization of processing parameters of the model

4.13 Microbial analyses of the juices

4.14 Cytotoxicity study

4.15 Flow cytometry analysis using RNase/Propidium iodide

4.16 DNA damage study using single cell gel electrophoresis (SCGE) assay (Comet assay)

4.17 Expression of oncogenes in HepG2 cell lines using reverse transcription-polymerase chain reaction (RT-PCR) method

5 DISCUSSION

5.1 Optimization of product formulation and processing parameters of high quality Centella asiatica and Strobilanthes crispus juices using response surface methodology (RSM)

5.2 Cytotoxicity study

5.3 Apoptosis induction by flow cytometry analysis and comet Assay

5.4 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) of oncogenes

6 CONCLUSION AND FUTURE RESEARCH RECOMMENDATION

6.1 Conclusion

6.2 Future research recommendations

REFERENCES

APPENDICES

BIODATA OF THE AUTHOR

LIST OF PUBLICATIONS