ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES AND IN VITRO CANCER CHEMOPREVENTIVE EFFECTS OF AVERRHOA BILIMBI L. EXTRACT ON HUMAN BREAST AND CERVICAL ADENOCARCINOMA

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By

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ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES AND IN VITRO CANCER CHEMOPREVENTIVE EFFECTS OF *AVERRHOA BILIMBI* L. EXTRACT ON HUMAN BREAST AND CERVICAL ADENOCARCINOMA

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June 2012

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High consumption of fruits has been associated with a reduced risk of various degenerative diseases such as cancer. These protective properties are reckoned to be linked to the micronutrients and non-nutritive phytochemicals present in fruits. This study was conducted to investigate the nutritional compositions, total phenolic and flavonoid contents, antioxidant capacity, antioxidant vitamins, phenolic acid and flavonoid compounds, as well as anticancer potential of two types of fruits endemic to Malaysia: *Averrhoa bilimbi* (bilimbi) and *Averrhoa carambola* (carambola). Commonly consumed among the locals, these underutilized fruits have been popularly practiced as folk medicine since ancient times.
Results from nutritional compositions analyses showed that bilimbi possessed higher fat and total dietary fibre but comparable moisture, ash, carbohydrate and protein content with that of carambola. Results from Folin-Ciocalteu, aluminium chloride colourimetric assays and antioxidant vitamins analyses indicated a higher antioxidant vitamins and flavonoid content in bilimbi while carambola with higher total phenolic, β-carotene bleaching and DPPH radical scavenging assays suggested that carambola displayed stronger antioxidant capacity and was positively correlated with its total phenolic content.

The presence of non-nutritive phytochemicals in both fruits was evaluated by HPLC analysis based on the optimized extraction conditions performed through single-factor experiments. Analysis revealed that optimal extraction conditions were 80% ethanol at 65 °C for 8 hours in both fruit extracts. Phenolic compounds extracted by the optimal extraction conditions were subjected to HPLC analysis. Epigallocatechin and catechin were identified as the key flavonoids in bilimbi and carambola, respectively while sinapic acid and syringic acid were the predominant phenolic acid in bilimbi and carambola, respectively.

Cytotoxic and anticancer potential of bilimbi and carambola ethanolic extracts were examined on various human cancer cell lines (breast, cervical, colon, liver and ovarian adenocarcinoma) as well as non-malignant Chang Liver cell line. Findings from MTS assay implied a 50% growth inhibitory effect induced by bilimbi extract on cervical cancer cell line (HeLa) and non-hormone dependent breast cancer cell line (MDA-MB-
231) at 70 and 90 µg/ml, respectively without any cytotoxic effect on non-malignant Chang Liver cell line. In contrast, carambola extract was unable to exert any growth inhibitory effect even concentration was increased up to 200 µg/ml. Further investigations to determine apoptogenic potential of bilimbi extract revealed typical morphological features of apoptosis as well as formation of ladder-like pattern in bilimbi-treated HeLa and MDA-MB-231. Apoptosis and antiproliferative effects were further evidenced with the increased in sub G1 population and accumulation of cells with a 2N DNA content in cell cycle analysis, suggesting perturbation at G0/G1 checkpoint. BrdU incorporation cell proliferation assay indicated a decreased in cells synthesis (S phase). Released of cytochrome c coupled with upregulation of caspase-3/7 and caspase-9 expression indicated that molecular mechanism in the induction of apoptosis by bilimbi on HeLa and MDA-MB-231 was through mitochondria-mediated intrinsic pathway, which also involve upregulation of pro-apoptotic Bax protein and downregulation of anti-apoptotic Bcl-2 protein. Upregulation of tumour suppressor p53 protein was detected in HeLa upon exposure to bilimbi, as opposed to MDA-MB-231 which was found to be independent of p53. Hence, bilimbi induced apoptosis through a p53-dependent mitochondrial pathway in HeLa and a p53-independent mitochondrial pathway in MDA-MB-231.

Based on the antioxidant and phytochemicals properties as well as anticancer potential, bilimbi offers a promising candidate for cancer chemoprevention strategy. As a natural product, bilimbi is potentially safe and beneficial for pharmaceutical and health industries worth further research and investigations.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KANDUNGAN ANTIOXIDAN DAN FITOKIMIA DAN KESAN IN VITRO KEMO PENCEGAHAN KANSER EKSTRAK AVERRHOA BILIMBI L. PADA ADENOKARSINOMA PAYUDARA DAN SERVIK MANUSIA

Oleh

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Keputusan analisis komposisi pemakanan menunjukkan bahawa bilimbi memiliki lemak dan serat yang tinggi tetapi kandungan air, abu, karbohidrat dan protein yang standing dengan carambola. Keputusan kaedah Folin-Ciocalteu, ujian kolorimetrik aluminium klorida dan vitamin antioksida analisis menunjukkan bahawa bilimbi mengandungi vitamin antioksida dan flavonoid yang lebih tinggi manakala manakala carambola mengandungi jumlah fenolik yang lebih tinggi. Ujian pelunturan β-karotena dan pemerangkapan radikal DPPH mencadangkan bahawa carambola memaparkan kapasiti antioksidan yang kuat dan berkait positif dengan jumlah kandungan fenolik.

Kehadiran fitokimia dalam kedua-dua buah-buahan dinilai oleh analisis HPLC berdasarkan keadaan pengekstrakan optima yang ditentukan melalui uji kaji satu faktor. Analisis menunjukkan bahawa keadaan pengekstrakan optima adalah 80% etanol pada 65 °C selama 8 jam pada kedua-dua ekstrak buah-buahan. Sebatian fenolik kemudian diekstrak dalam keadaan pengekstrakan optima melalui analisis HPLC. Epigallocatechin dan catechin masing-masing dikenal pasti sebagai flavonoid utama dalam bilimbi dan carambola manakala asid sinapic dan asid syringic masing-masing dikenal pasti sebagai asid fenolik utama dalam bilimbi dan carambola.

Potensi sitotoksik dan anti kanser ekstrak etanol bilimbi dan carambola disaring pada beberapa sel kanser manusia (adenokarsinoma payudara, serviks, kolon, hati dan ovari) serta sel normal Chang Liver. Keputusan analisis MTS menunjukkan bahawa ekstrak bilimbi merencat pertumbuhan sel kanser serviks (HeLa) dan payudara (MDA-MB-231) sebanyak 50% masing-masing dengan nilai 70 dan 90 µg/ml tanpa kesan sitotoksik ke
atas sel normal Chang Liver. Sebaliknya, ekstrak carambola tidak mampu merencat
d pertumbuhan sebarang sel kanser walaupun kepekatan ekstrak ditingkatkan sehingga
200 µg/ml. Siasatan lanjut untuk menentukan potensi apoptosis ekstrak bilimbi
menunjukkan bahawa kesan ekstrak bilimbi ke atas HeLa dan MDA-MB-231
menunjukkan ciri-ciri morfologi lazim apoptosis serta pembentukan pola DNA ladder.
Kesan apoptosis dan anti proliferasi juga dibuktikan melalui peningkatan fasa sub G1
dan peningkatan sel dengan kandungan 2N DNA dalam analisis kitaran sel yang
menyebabkan pembantutan proses kitaran sel di fasa G0/G1. Ujian sel proliferasi BrdU
menunjukkan pengurangan sintesis sel (fasa S). Pengaktifan sitokrom c serta
peningkatan caspase-3/7 dan caspase-9 menunjukkan bahawa mekanisme induksi
apoptosis oleh bilimbi pada HeLa dan MDA-MB-231 di peringkat molekul adalah
melalui lautan intrinsik mitokondria yang juga melibatkan peningkatan protein pro
apoptosis Bax dan penurunan protein anti apoptosis Bcl-2. Pendedahan HeLa kepada
ekstrak bilimbi menyebabkan peningkatan protein tumour suppressor p53, sebaliknya,
induksi apoptosis oleh bilimbi pada MDA-MB-231 didapati tidak melibatkan protein
tumour suppressor p53. Oleh itu, induksi apoptosis oleh bilimbi pada HeLa adalah
bergantung kepada p53 dan melalui lautan intrinsik mitokondria manakala pada MDA-
MB-231 adalah tidak bergantung kepada p53 dan melalui lautan intrinsik mitokondria.

Berdasarkan ciri-ciri antioksidan dan fitokimia serta potensi anti kanser, bilimbi
berupaya digunakan dalam strategi kemo pencegahan kanser. Sebagai produk
semulajadi, bilimbi adalah selamat dan bermanfaat untuk industri farmaseutikal dan
kesihatan serta amat sesuai untuk penyelidikan dan siasatan lanjut.
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"Success is not final, failure is not fatal; it is the courage to continue that counts”

~ Winston Churchill ~

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I certify that a Thesis Examination Committee has met on 11 June 2012 to conduct the final examination of Yan See Wan on her thesis entitled “Antioxidant and Phytochemical Properties and In Vitro Cancer Chemopreventive Effects of Averrhoa bilimbi L. Extract on Human Breast and Cervical Adenocarcinoma” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1988. The Committee recommends that the students be awarded the degree of Doctor 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.

YAN SEE WAN

Date: 11 June 2012
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