

**CYTOTOXIC AND ANTI-HUMAN PAPILLOMAVIRUS
ACTIVITIES IN SELECTED VEGETABLES**

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**FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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CYTOTOXIC AND ANTI-HUMAN PAPILLOMAVIRUS ACTIVITIES IN SELECTED VEGETABLES

ABSTRACT

An appropriate way to control cancer is chemoprevention as a large number of compounds have been observed to prevent the occurrence of cancer and variety of mechanisms exists for producing such protective effects. Numerous epidemiological studies have shown that diets rich in vegetables are associated with a decreased risk of cancer. Chemoprevention involves the use of natural or laboratory made substances to prevent cancer or reduce risk cancer.

The E6 oncoprotein from oncogenic Human papillomaviruses (HPVs) has exhibit functions in tumorigenesis, regulation of transcriptions and apoptosis. E6 product from oncogenic viral type like HPV 16 can bind the tumor suppressor p53 and promotes its degradation. In the present study, 80 crude methanol and water extracts obtained from 40 selected vegetables were screened and evaluated for their cytotoxic and anti-human papillomavirus 16 E6 activities.

The cytotoxic effect of the varying concentrations of both methanol and water vegetable extracts (0, 1, 10, 25, 50 and 100 $\mu\text{g/ml}$) against cervical cancer-derived, HeLa and CaSki cells and normal cell-derived, MRC5 (human lung fibroblast) cells were evaluated using the *in vitro* neutral red cytotoxic assay. The IC_{50} values for each vegetable extract was determined. The results indicated that the percentage of killing increased with the increasing concentration of extracts tested. Overall, the crude methanol vegetable

extracts showed greater inhibition against the growth of cervical cancer-derived cell lines, HeLa and CaSki cells. The methanolic vegetable extracts exerted signs of cell-type selectivity against cervical cancer-derived cell lines where 29 extracts were active against CaSki. However, only 7 extracts were effective in inhibiting the growth of HeLa cells.

Twenty vegetable extracts, both methanol and water extracts of *Asparagus officinalis*, *Beta vulgaris*, *Ipomea batatas*, *Manihot esculentus*, *Pachyrrizus erosus*, *Petroselinum crispum*, *Phaseolus vulgaris*, *Portulaca oleracea*, *Sesbania grandiflora*, and *Solanum tuberosum* were analyzed qualitatively at various concentrations (0, 1, 10, 25, 50 and 100 µg/ml) for their anti-HPV 16 E6 in HPV 16 containing, cervical cancer-derived cell line, CaSki. Immunocytochemistry technique was carried out to analyze the expression of HPV 16 E6 oncoprotein in CaSki cells treated with varying amounts of the vegetable extracts. The results indicated that methanolic extracts of *Phaseolus vulgaris* suppression of E6 oncoprotein in CaSki cells which increased in cells treated independently with increasing concentrations of the vegetable extracts. Generally, the crude water extracts were most effective in suppressing the expression of HPV 16 E6 oncoprotein in CaSki cells.

The results of the present study indicated that out of the 40 selected vegetables screened, *Portulaca oleracea* (*Beremi*) and *Beta vulgaris* (*Beetroot*) showed significant cytotoxic effects and anti-HPV 16 E6 activities in CaSki cells. These findings suggest that some of the vegetables may tested have great potential to be exploited for effective chemoprevention and as anti-HPV 16 E6 agent in the control of cervical cancer.

ABSTRAK

**AKTIVITI SITOTOKSIK DAN ANTI-VIRUS PAPILLOMA MANUSIA DALAM
SAYURAN TERPILIH**

Cara yang paling berkesan untuk mengawal kanser ialah kemopenghalang dimana banyak sebatian yang telahpun dinilai untuk menghalang terjadinya kanser dan bermacam-macam mekanisma hadir untuk menghasilkan kesan pelindung. Kebanyakan kajian epidemiologi menunjukkan bahawa pemakanan yang kaya dengan sayuran mempunyai kaitan dengan penurunan risiko menghidapi kanser. Kemopenghalang melibatkan penggunaan bahan semulajadi atau bahan yang dihasilkan di makmal untuk tujuan menghalang pertumbuhan kanser atau mengurangkan risiko kanser..

Oncoprotein E6 dari HPVs onkogenik mempamerkan fungsi dalam tumorigenesis, mengawal penyalinan dan apoptosis. Hasilan E6 dari jenis viral onkogenik seperti HPV 16 boleh mengikat penindas tumor p53 dan menggalakan kemusnahan. Dalam kajian ini, 80 ekstrak mentah mentanol dan air suling didapati dari 40 jenis sayuran terpilih diskrim dan dinilai untuk aktiviti sitotoksik dan anti-human papillomavirus 16 E6 manusia.

Kesan sitotoksik pada kepekatan yang belainan bagi ekstrak sayuran pada kepekatan-kepekatan berbeza (0, 1, 10, 25, 50 and 100 $\mu\text{g}/\text{ml}$) terhadap sel terbitan kanser sekvik, HeLa, CaSki dan sel normal terbitan sel MRC5 (fibrolast jantung manusia) dinilai menggunakan asai sitotosik *in vitro* neutral merah assei sitotoksik. Nilai IC_{50} bagi setiap ekstrak sayuran ditentukan. Keputusan yang didapati menunjukkan bahawa kenaikan peratus kematian meningkat dengan peningkatan kepekatan ekstrak yang dikaji. Secara

keseluruhannya, ekstrak sayuran mentah metanol menunjukkan pemilihan pada jenis sel terbitan dari leluhur sel kanser serviks, dimana 29 ekstrak adalah aktif terhadap sel CaSki. Walaubagaimanapun, hanya 7 ekstrak yang efektif merencat pertumbuhan sel HeLa.

Dua puluh ekstrak sayuran iaitu ekstrak methanol dan air bagi sayuran asparagus, bit, keledak, ubi kayu, sengkawang, parsli, kacang putih, beremi, turi dan ubi kentang telah dianalisis secara kualitatif pada kepekatan yang berlainan (0, 1, 10, 25, 50 and 100 µg/ml) bagi anti-HPV 16 E6 dalam sel terbitan kanser servik, yang mengandungi HPV 16. Teknik immunositokimia yang telah digunakan untuk menganalisis E6 oncoprotein HPV 16 dalam CaSki yang telah dirawat dengan pelbagai jenis ekstrak sayuran. Keputusan menunjukkan bahawa bagi ekstrak methanol kacang putih peningkatan penindasan oncoprotein E6 di dalam sel CaSki yang dirawat tidak bergantung kepada peningkatan kepekatan ekstrak sayuran. Secara umumnya, ekstrak air paling efektif dalam penindasan E6 oncoprotein HPV 16 dalam sel CaSki.

Keputusan ini menunjukkan bahawa *Portulaca oleracea* (beremi) dan *Beta vulgaris* (bit) mempunyai kesan aktiviti sitotoksik dan anti-HPV 16 E6 dalam sel CaSki yang signifikan. Keputusan ini mencadangkan bahawa sesetengah sayuran mempunyai potensi yang baik untuk dikaji sebagai agen anti kanser yang efektif dan agen anti-HPV 16 E6 dalam mengawal kanser servik.

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LIST OF ABBREVIATIONS

ATCC	American Tissue Culture Collection
CIN	cervical intraepithelial neoplasia
DAB	carbon dioxide
DMSO	3-diaminobenzidine-tetrahydrochloride
E	early Region
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetra acetic acid
ELISA	enzyme-linked immunosorbent assay
FCS	foetal calf serum
G	gramme
hr	hour
H ₂ O ₂	hydrogen peroxide
HRP	horseradish peroxidase
HEPES	N-2-Hydroxyethyl-Piperazine-N-2-Ethane-Sulfonoc
HPV	human papillomavirus
HSIL	high grade squamous intraepithelial lesion
IC	inhibition concentration
kg	kilogramme
KH ₂ PO ₄	potassium dihydrogen orthophosphate
L	late region
Lab	laboratory
M	molar
mg	milligramme
min	minutes
ml	millilitre
mm	millimeter
NaCl	sodium chloride
NaHCO ₃	sodium bicarbonate
Na ₂ HPO ₄	sodium phosphate anhydrous
nM	nanometer
No.	number
NR	Neutral Red
°C	degree Celsius
OD	optical density
ORF	open reading frames
PBS	phosphate buffered saline
rpm	rotation per minutes
RPMI	Rosewell Park Memorial Institute
UV	ultraviolet
URR	upstream regulatory region
w/v	weight per volume
wt	weight
%	percentage
µg	microgramme
µl	microlitre
µm	micrometer

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