# Cell survival Inhibitory Effect of *Raphanus sativus* v. *caudatus* Alef Extracts against Non-resistant HepG2 and Resistant HepG2 Hepatocellular Carcinoma

# ผลของสารสกัดจาก *Raphanus sativus* v. *caudatus* Alef ต่อการยับยั้งการเจริญเติบโตของเซลล์มะเร็งตับ HepG2 และเซลล์ HepG2 ที่ดื้อยา

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**ABSTRACT**

The determination of cell survival inhibitory effect is also important for the anti-cancer treatment. In this study *Raphanus sativus* v. *caudatus* Alef (RS) extracts has been discovered for its cytotoxic activity against hepatocellular carcinoma (HepG2) as well as the resistant HepG2 cells. Moreover the inhibition of cell survival in the period after removing the treatment was also investigated. The neutral red assay was used in the determination of percentage of cell viability. The results were compared with chemotherapeutic drugs; cisplatin and melphalan. The well-known isothiocyanate chemopreventive compounds; sulforaphene and sulforaphane were also been used in the study. After the removal of the treatment, the cell viability was observed for 3 days. The RS extracts showed the delay of resistant HepG2 cell recovery. RS reveals not only cytotoxic effect but also the cell survival efficacy.

**บทคัดย่อ**

การตรวจสอบผลการยับยั้งการเจริญเติบโตของเซลล์มะเร็งเป็นหนึ่งในส่วนสำคัญของยารักษามะเร็ง ในการศึกษานี้พบว่าสารสกัด *Raphanus sativus* v. *caudatus* Alef (RS) มีความเป็นพิษต่อเซลล์มะเร็งตับ (HepG2) และเซลล์ดังกล่าวที่มีคุณสมบัติดื้อยา และยังพบว่าการเจริญเติบโตของเซลล์ถูกยับยั้งหลังจากการนำสารทดสอบออก โดยใช้วิธีทดสอบ neutral red และทำการเปรียบเทียบผลการทดสอบกับยารักษามะเร็งสองชนิดคือ cisplatin และ melphalan และสารประกอบกลุ่มไอโซไธโอไซยาเนตสองชนิดคือ ซัลโฟราฟีนและซัลโฟราเฟน จากการทดสอบหลังนำสารทดสอบออกเป็นเวลาสามวัน พบว่าสารสกัดสามารถชะลอการเจริญเติบโตของเซลล์ HepG2 ที่ดื้อยา จากการศึกษานี้พบว่าสารสกัด RS ไม่เพียงเป็นพิษต่อเซลล์มะเร็ง แต่ยังช่วยยับยังการเจริญเติบโตของเซลล์มะเร็งหลังจากนำสารทดสอบออก

**Key Words:** Cell survival inhibition, Cytotoxicity, HepG2, Isothiocyanate, *Raphanus sativus* v. *caudatus* Alef, Resistant HepG2

**คำสำคัญ:** การยับยั้งการเจริญเติบโต ความเป็นพิษต่อเซลล์ เซลล์ HepG2 ไอโซไธโอไซยาเนต *Raphanus sativus* v. *caudatus* Alef เซลล์ HepG2 ที่ดื้อยา

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## Introduction

Hepatocellular carcinoma was the third cancer-related deaths in the world. There is still high rate of failure in anti-cancer chemotherapy causes by the development of drug resistance (Yang et al., 2010). Widely consumed broccoli, cabbage, radish, and wasabi—belong to Brassicaceae family—were reported to possess health promoting benefits (Jahangir et al., 2009; Björkman et al., 2011). They consisted of useful fibers, vitamins, and minerals and also the secondary metabolites such as polyphenols, glucosinolates and isothiocyanates (ITCs) (Jahangir et al., 2009; Björkman et al., 2011; Cartea et al., 2011). Sulforaphane is one of the isothiocyanate compound widely known to have anticancer activity through apoptosis pathway (Kristjansdottir et al., 2012).

## Objective of the study

This study were purposed to determine cytotoxicity and also the inhibition of cell survival effect of Thai native plant in Brassicaceae family—*Raphanus sativus* L. var. *caudatus* Alef against HepG2 cancer cell and its resistant model.

## Methodology

### Materials

The analytical grade dichloromethane was purchase from Fisher scientific, UK. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO (Life technologies, California, USA). Neutral red dye was purchased from Sigma Chemicals Co. (Missouri, USA). L-sulforaphene was purchased from Enzo Life Science (NY, USA). D,L-Sulforaphane was purchased from Calbiochem (Merck, Darmstadt, Germany).

### Sample preparation and extraction

Thai rat-tail radish or *Raphanus sativus* L. var. *caudatus* Alef (RS) was harvested at the 6$^{th}$ week. Fresh stem and pod parts were used for the extraction following the method as described in Pocasap et al. (2013). Fresh RS was blended with deionized water for 30 min. and left for autolyzing at room temperature for another 2 hr. Then the homogenate was filtered to collect filtrate for further liquid-liquid extraction with dichloromethane. The solvent was removed by rotary evaporator yielding the dry crude extract.

### Determination of cell viability

In this study HepG2 cell lines and its resistant model were used. The resistant cell lines were generated by in vitro induction by stepwise increase of cisplatin concentrations. The cell viability was determined by neutral red assay (Machana et al., 2011). Briefly, in order to determine the cell cytotoxicity, cell density of $3\times10^4$ cells/ well was treated with drugs, pure isothiocyanates (ITCs) and RS extracts at various concentrations. After 24 hr of treatment, cells were washed with 1× PBS and added with NR solution. After 2 hr, cells were lysed and the absorbance of NR was detected by a dual-wavelength UV spectrometer at 537/650 nm. A plot of % cytotoxicity versus concentrations of tested compounds was used to extrapolate the concentration possessing 50% cytotoxicity ($IC_{50}$).

In order to determine the cell survival inhibition, cell were treated with $1\times IC_{50}$. After 24 hr, the treatments were removed and replaced with media. At 24 hr interval, cells of each treatment set were performed for NR assay. The cell survival was investigated for 3 days. The percentages of cell survival were calculated compared to Day 0 of treatment removal.

## Results

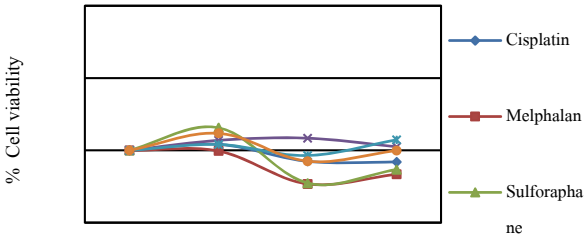
Cell cytotoxicity was determined in order to obtain an $IC_{50}$ for using in cell survival determination. Table 1 shows the $IC_{50}$ of all treatments against HepG2 and resistant HepG2. The results showed the higher $IC_{50}$ values of all treatments in the resistant HepG2 than in HepG2 cells.

**Table 1** Cytotoxicity of treatment against HepG2 and resistant HepG2

| Treatment | $IC_{50}$ (µg/ml) | |
|---|---|---|
| | **HepG2** | **Resistant HepG2** |
| Cisplatin | 65.06±0.56 | 117.97±1.98 |
| Melphalan | 91.28±9.08 | 101.80±1.72 |
| Sulforaphane | 13.60±0.9 | 16.72±0.09 |
| Sulforaphene | 13.96±0.34 | 25.43±0.66 |
| RS pod | 57.26±2.09 | 247.47±1.68 |
| RS stem | 172.72±3.19 | 401.69±25.46 |

The results in Figure 1 showed the trend of cell viability rate after the treatments were removed. It was found that in resistant HepG2 cells, cell viability was recovered in Day 3 but not in non-resistant HepG2 cells. Among all treatments, RS pod minimized the percentage of cell survival the most, followed by melphalan and RS stem. The results also revealed the higher anti-cell survival of crude RS extracts than the pure ITCs compounds.

A

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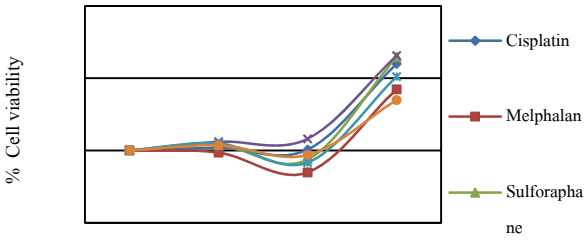


**Figure 1** HepG2 (A) and resistant HepG2 (B) cell survival after 3 days; Day 0 means immediately after treatment removal, Day 1, 2, and 3 means 24 hr, 48 hr and 72 hr after removal of the treatments, respectively

## Conclusions

In conclusion, the present study determined the effect of *Raphanus sativus* v. *caudatus* Alef (RS) extracts not only as the chemopreventive agent but also delayed the cell survival of HepG2. Our study provides the usefulness of Thai native vegetable *Raphanus sativus* v. *caudatus* Alef (RS). The detailed mechanisms of these claims should be further investigated.

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**References**

Yang L, Liu X, Lu Z, Chan J. Y-W, Zhou L, Fung K-P , Wu P, Wu S. Ursolic acid induces doxorubicin-resistant HepG2 cell death via the release of apoptosis-inducing factor. Cancer Letters.298(1) 2010, 128–138

Jahangir M, Kim HK, Choi YH, Verpoorte R. Health-Affecting Compounds in Brassicaceae. *Comprehensive Reviews in Food Science and Food Safety*. 2009, 8: 31-43.

Björkmana M, Klingen I, Birch ANE, Bones AM, Bruce TJA, Johansen TJ, Meadow R, Mølmann, Seljåsen R, Smart LE, Stewart D. Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. *Phytochemistry* 2011, 72: 538-556.

Kristjansdottir K, Kim K, Choi JS, Horan TC, Brard L, Moore RG, Singh RK. 7-Methyl indole ethyl isothiocyanate causes ROS mediated apoptosis and cell cycle arrest in endometrial cancer cells. *Gynecologic Oncology* 2012, 126: 252-258.

Machana S, Weerapreeyakul N, Barusrux S, Nonpunya A, Sripanidkulchai B, Thitimetharoch T. Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line. *Chinese Medicine* 2011: 6: 39.

Pocasap P, Weerapreeyakul N, Barusrux S. Cancer preventive effect of Thai rat-tailed radish (*Raphanus sativus* L. var. *caudatus* Alef). *Journal of Functional Foods* 2013, 5: 1372-1381