

# Cytotoxic Activity of *Toona sinensis* on Human Lung Cancer Cells

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## **Abstract**

*Toona sinensis* Roem (*Cedrela sinensis* A. Juss.) is a broadleaf tree and was widely used as a vegetable in China for thousand years. This plant can be used medicinally to cool down heat, to detoxify, to kill insects, as treatment of eye infections and enteritis etc. The previous study showed that the crude extract from the leaves of *T. sinensis* exerts potent antiproliferative effect on A549. In the present work, we examined the cytotoxic activity of methanol extracts from *T. sinensis* leaves, stem, barks, wood, root, and the aqueous crude extract (TSL-1) for three types of NSCLC, H441, H661, and H520 cells. We also tested major isolates of *T. sinensis* to evaluate its anticancer bioactive principles. The result showed that the MeOH extract of *T. sinensis* leaves and TSL-1 possessed significantly cytotoxic activity against the three types of NSCLC ( $P < 0.01$ ). Gallic acid showed the cytotoxicity for H441, H661, and H520 by 77%, 67%, and 79% at the concentration of 0.5 mg/ml, respectively. Ethyl gallate showed cytotoxic activity similar to gallic acid, but with rather weak activity. The results suggested that plant phenol, gallic acid and ethyl gallate, may play an important role in the cytotoxic activity of *T. sinensis*.

**Keywords** *Toona sinensis*, Cytotoxic activity, Gallic acid, Human Lung Cancer Cells

## 1. Introduction

*Toona sinensis* Roem. (*Cedrela sinensis* A. Juss ), a member of Meliaceae, is a deciduous tree which grows in southern Taiwan and is also widely be cultivated in China. A number of compounds including retinoid, vitamins B and C, o-coumaric acid, kaempferol, methyl gallate, quercetin, afzelin, quercitrin, isoquercitrin, rutin, cedrellin and phytol derivatives have been isolated from this plant (Park et al., 1994; Park et al., 1996; Luo et al., 2000) The leaves of *T. sinensis* are edible and have been used as oriental medicine for treating enteritis, dysentery and itch. No irreversible side effects were observed after treatment (Park et al., 1993; Edmonds and Staniforth, 1998). Our previous studies also indicated that the crude extracts from the leaves of *T. sinensis* exerts potent antiproliferative effect on A549 human lung cancer cells and show no significant cytotoxic effect on primarily cultured human foreskin fibroblasts or MRC-5 human lung fibroblasts (Chang et al., 2002). Lung cancer is a world problem and one of the most lethal malignancies in many countries. In addition, many lung cancer cells showed significant resistance to chemotherapeutic drugs. Therefore, development of new therapeutic drugs, an adjuvant in combination with chemotherapeutic drugs, or even useful chemo preventive agent for lung cancer is clinically important work.

As a part of our continuing investigation on the phytochemical and bioactive principles of *T. sinensis*, some known compounds including methyl gallate, gallic acid, kaempferol, quercetin, quercitrin, rutin, catechin, epicatechin, oleic acid, palmitic acid, linoleic acid, linolenic acid, a mixture of  $\beta$ -sitosterol and stigmasterol, and  $\beta$ -sitosteryl-glucoside, were isolated and identified from this plant (Hsieh et al., 2004) Since A549 is one of the non-small-cell lung cancer (NSCLC) types, it is very interesting

to know whether *T. sinensis* can show the anticancer effect on all three types of NSCLC or not. In the present work, we examined the cytotoxic activity of methanol extracts from *T. sinensis* leaves, stem, barks, wood, root, and the aqueous crude extract (TSL-1) for three types of NSCLC, H441 cells (adenocarcinoma), H661 cells (large cell carcinoma) and H520 cells (squamous cell carcinoma). We also tested major isolates including gallic acid, ethyl gallate, and methyl gallate to evaluate the anticancer bioactive principles of *T. sinensis*.

## **2. Materials and method**

### *2.1. Plant material*

*Toona sinensis* Roem. (*Cedrela sinensis* A. Juss.) were collected from Fooyin University, Kaohsiung Hsien, Taiwan in September, 2001. A voucher specimen was characterized by Dr. Horng-Liang Lay, Graduate Institute of Biotechnology, National Pingtung University of Science and Technology, Pingtung County, Taiwan and deposited in the Fooyin University, Kaohsiung Hsien, Taiwan.

### *2.2. General instrumental equipment*

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained in MeOH by using a JASCO V-530 spectrophotometer. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. The IR spectra were measured on a Hitachi 260-30 spectrophotometer. <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) spectra were obtained on a Varian NMR spectrometer. LRFABMS and LREIMS spectra were obtained with a JEOL JMS-SX/SX

102A mass spectrometer or a Quattro GC-MS spectrometer with a direct inlet system. High-resolution EIMS were measured on a JEOL JMS-HX 110 mass spectrometer. Si gel 60 (Merck, 230-400 mesh) was used for column chromatography, precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC and precoated Si gel plated (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. The spots were detected by spraying with 50% H<sub>2</sub>SO<sub>4</sub> and then heating on a hot plate.

### 2.3. Preparation of extracts and Isolates

The dried and powdered leaves (3 Kg), stem (1.5 Kg), barks (1.2 Kg), wood (30 Kg) and root (10 Kg) of *T. sinensis* were collected and extracted repeatedly with MeOH at room temperature to afford the crude MeOH extracts of leaves (588 g), stem (244 g), barks (115 g), wood (963 g) and root (120 g) for further study, respectively. The isolation procedure of the extracts was described in our previous study (Hsieh et al., 2004). The major constituents (as shown in Fig. 1) from the extracts of *T. sinensis* including, gallic acid (yield: 0.1% from dried leaves, 0.012% from root), ethyl gallate (yield: 0.03% from dried leaves, 0.01% from root), and methyl gallate (yield: 0.2% from dried leaves, 0.02% from root) were quickly collected and used in this study. We also prepared another aqueous crude extract (TSL-1), as previously described (Chang et al., 2002), and by adding 1000 ml water to 100g leaves then boiling until 100 ml remained for the test.

### 2.4. Cell culture

Human bronchial epithelial cells (BEAS-2B) was cultured in serum-free bronchial epithelial basal medium (BEBM, Clonetics) supplemented with bovine pituitary extract

(52 µg/ml), hydrocortisone (0.5 µg/ml), epidermal growth factor (0.5 ng/ml), epinephrine (0.5 µg/ml), transferrin (10 µg/ml), insulin (5 µg/ml), retinoic acid (0.1 ng/ml), triiodothyronine (6.5 ng/ml), gentamicin-1000 (50 µg/ml) and amphotericin-B (50 ng/ml). Additional the human lung carcinoma cell lines, H441, H661, and H520, were cultured in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/ml penicillin, 2 mM L-glutamine, 10 mM HEPES (N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid), and 1.0 mM sodium pyruvate. Cells were cultured as monolayer in standard plastic tissue culture dishes and flasks, and incubated in a humidified incubator in an atmosphere of 95% air, 5% CO<sub>2</sub> at 37°C. Then the cells were treated with the different extracts and isolates for 24h and harvested for analysis.

### *2.5. MTT cytotoxicity test*

The cytotoxicity assays were performed with the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] (MTT) method, using tetrazolium salt (Mosman, 1983; Denizot and Lang, 1986; Rahn et al., 1991, Vistica et al., 1991). BEAS-2B (2X10<sup>4</sup> cells/well), H441 (1X10<sup>4</sup> cells/well), H661 (4X10<sup>3</sup> cells/well), and H520 (1X10<sup>4</sup> cells/well) cells were seeded in 96-well plates and grown overnight. The cells were then treated with the different extracts (1 mg/ml) and isolates (0.5 mg/ml) for 24h. After incubation, media were replaced with 50 µl of MTT reagent (2 mg/ml) and incubated in a 5% CO<sub>2</sub> incubator at 37 °C for another 4 h. The cells were harvested in 50 µl of DMSO, and absorbance was measured at 540 nm by using an ELISA reader (Molecular Probes Inc., Eugene, OR).

## 2.6. Statistic

Results are expressed as mean  $\pm$  S.D and accompanied by the number of observations. A one-way analysis of variance (ANOVA) was used for multiple comparisons, and if there was significant variation between treatment groups, then the mean values for inhibitors were compared with those for control by Student's *t*-test, and *p* values of less than 0.05 were considered to be statistically significant.

## 3. Results and discussion

Repeated column chromatography of the MeOH extracts of *T. sinensis* on silica gel followed by reverse phase C-18, Sephadex LH-20, and preparative TLC led to the isolation of twenty compounds. These known compounds were identified as methyl gallate, ethyl gallate, gallic acid, kaempferol, kaempferol-3-*O*- $\beta$ -*D*-glucoside, quercetin, quercetin-3-*O*- $\beta$ -*D*-glucoside, quercitrin, rutin, catechin, epicatechin,  $\beta$ -sitosterol, stigmasterol,  $\beta$ -sitosteryl-  $\beta$ -*D*-glucoside, stigmasteryl-  $\beta$ -*D*-glucoside, oleic acid, palmitic acid, linoleic acid, linolenic acid, and phytol by comparing spectral data (UV, MS,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ ) with those previously reported (Park et al., 1994; Park et al., 1996, Hsieh et al., 2004). The crude extracts of *T. sinensis* leaves, stem, barks, wood, root, and TSL-1 were tested for their cytotoxic activity on human lung cancer cells. The results are summarized in Table 1. The data showed that the MeOH extract of *T. sinensis* leaves and TSL-1 possessed significantly cytotoxic activity against the three types of NSCLC at the concentration of 1 mg/ml. The extract of barks also significantly inhibited H661 and H520 cells growth. Among these extracts only the stem of *T. sinensis* did not show any

cytotoxic activity on cell growth of BEAS-2B and NSCLC. In addition, the cytotoxic effect of major constituents isolated from MeOH extracts of *Toona sinensis* leaves were shown in Fig. 2. The antiproliferative effect of gallic acid, ethyl gallate, and methyl gallate on three types of NSCLC revealed different results. Gallic acid (0.5 mg/ml) showed the cytotoxicity for H441, H661, and H520 by 77%, 67%, and 79%, but for BEAS-2B only by 45%, respectively. Ethyl gallate showed cytotoxic activity similar to gallic acid, but with rather weak activity. And methyl gallate did not show any cytotoxic effect on cell growth of NSCLC at the concentration of 0.5 mg/ml.

Our previous study indicated that the aqueous crude extract of leaves from *T. sinensis* (TSL-1) exerts potent antiproliferative effect on A549 lung cancer cells. Further study revealed that the TSL-1 effectively blocked cell cycle progression by inhibiting the expression of cyclin D1 and E and led to apoptotic cell death in A549 cells (Chang et al., 2002). In present study, the results demonstrate that both of the TSL-1 and MeOH extract of *T. sinensis* leaves possess the antiproliferative effect on all three cell types of NSCLC. Between the two extracts, the MeOH extract of leaves showed a higher cytotoxic activity than that of TSL-1. This may be due to the different solvent and procession used to extract the different constituents having cytotoxic activity. Consequently, further chemical and pharmacological investigations were carried out and the major isolates including, gallic acid, ethyl gallate, and methyl gallate were quickly obtained at present time to evaluate the anticancer bioactive principles of *T. sinensis*. These major isolates, however, showed lesser cytotoxicity against BEAS-2B than NSCLC. And all NSCLC cells death occurred after gallic acid and ethyl gallate treatment at the concentration of 0.5 mg/ml. Gallic acid is a naturally occurring plant phenol and is known to show

biological and pharmacological activity (Mirvish et al., 1975; Huangh et al., 1985). Recently, biochemical research has demonstrated that gallic acid can induce selective cell death in some cancer cells (Inoue et al., 1995). The results showed that plant phenol, gallic acid and ethyl gallate, may play an important role in the cytotoxic activity of *T. sinensis*. Further studies in experimental animals will be helpful in evaluating the safety and anticancer effect of *T. sinensis in vivo*. Our data also suggests that TSL-1 may be used as an adjuvant in combination with chemotherapeutic drugs for lung cancer treatment or as a useful agent for chemoprevention.

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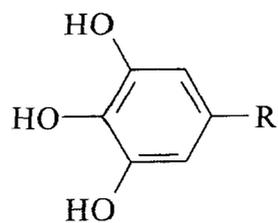
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Fig 1 Structures of major constituents isolated from the extracts of *Toona sinensis*



**Gallic acid**    R=COOH  
**Methyl gallate**    R=COOCH<sub>3</sub>  
**Ethyl gallate**    R=COOC<sub>2</sub>H<sub>5</sub>

Table 1. Cytotoxic effect of extracts of various parts of *Toona sinensis* on cell growth of human bronchial epithelial cells and human non-small-cell lung cancer cells analyzed by MTT assay.<sup>1</sup>

	Viable Cells (% of control)			
	H441	H661	H520	BEAS-2B
Leaves	67.5 ± 4.0**	42.0 ± 2.9**	48.8 ± 3.0**	43.2 ± 1.5**
Stem	105.8 ± 4.4	102.0 ± 3.4	111.1 ± 5.3	96.0 ± 2.4
Barks	98.3 ± 3.5	65.7 ± 4.5**	81.9 ± 5.3**	61.1 ± 1.0**
Wood	109.6 ± 4.5	93.3 ± 3.9	106.4 ± 4.0	50.1 ± 1.6**
Root	106.4 ± 4.3	94.5 ± 4.5	109.6 ± 5.8	72.8 ± 1.5**
FSL-1	86.1 ± 5.0*	55.4 ± 2.2**	76.0 ± 6.3**	73.4 ± 1.4**

<sup>1</sup> All values are mean ± S.D. (n = 10).

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

Fig 2 Cytotoxic effect of TSL-1 and major compounds isolated from MeOH extracts of *Toona sinensis*.

