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Phytochemical analysis of the triterpenoids with cytotoxicity and QR inducing properties from the total tea seed saponin of *Camellia sinensis*

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ABSTRACT

The tea seed triterpene saponin (TS) from *Camellia sinensis* was found to exhibit better antitumor activity *in vivo* in S180 implanted ICR mice and QR inducing activity for hepa lclc7 cells respectively compared with the total tea seed saponin (TTS), hydrolysate of the TTS and tea seed flavonoid glycosides (TF). By bioassay-guided isolation, the TS fraction was separated and seven major components were purified and identified as theasaponin E1 (**1**), theasaponin E2 (**2**), theasaponin C1 (**3**), assamsaponin C (**4**), theasaponin H1 (**5**), theasaponin A9 (**6**), and theasaponin A8 (**7**), among which compounds **4** and **5** were isolated from this genus for the first time. The antitumor bioassay of the isolated compounds showed that compounds **1**, **2** and **3** exhibited potential activities against the human tumor cell lines K562 and HL60. Furthermore, compound **1** (the major constituent with a mass content of over 1%) showed significant QR inducing activity with an IR value of 4.2 at 4 μg/ml. So it can be concluded that tea seed especially the compound **1** (theasaponin E1) could be used as an antitumor agent and a chemoprevention agent of cancer. The preliminary structure–activity relationship in the anti-tumor activity and QR inducing activity of tea saponins was discussed briefly.

1. Introduction

Camellia sinensis is a tea plant that is widely cultivated in China and Japan, and a plant species whose leaves and leaf buds are used to produce tea. Tea seed was recorded as an herb with expectorant, antitussive and anti-asthmatic effects in the Compendium of Materia Medica. The seeds of this tea plant (*C. sinensis*) are known to contain saponin constituents with an insectifuge activity and its crude saponin fraction has been used as a surface-active agent. Previous chemical investigation on the seeds of *C. sinensis* demonstrated the presence of acylated polyhydroxy oleanic-type triterpenoids with gastroprotective effect [1–5]. It has been reported that oleanic-type triterpenoids are an important resource of antitumor drug. So, we carried out research work on the antitumor activities and chemoprevention function of cancer of this tea seed.

The present paper described the bioactivity-guided fractionation of the crude ethanol extract of seeds of *C. sinensis*, leading to the isolation of seven theasaponins: theasaponin E1 (**1**), theasaponin E2 (**2**), theasaponin C1 (**3**), assamsaponin C (**4**), theasaponin H1 (**5**), theasaponin A9 (**6**), and theasaponin A8 (**7**), among which compounds **4** and **5** were isolated from this genus for the first time (Fig. 1). The fraction of total tea seed saponin shows anti-tumor activity in S180 implanted ICR mice and the isolated theasaponins exhibited different tumor inhibitory effects against human tumor cell lines, respectively. At the same time, the quinone reductase inducing activities were also estimated to reveal the possibility of tea seed for use as a chemoprevention agent of tumor.

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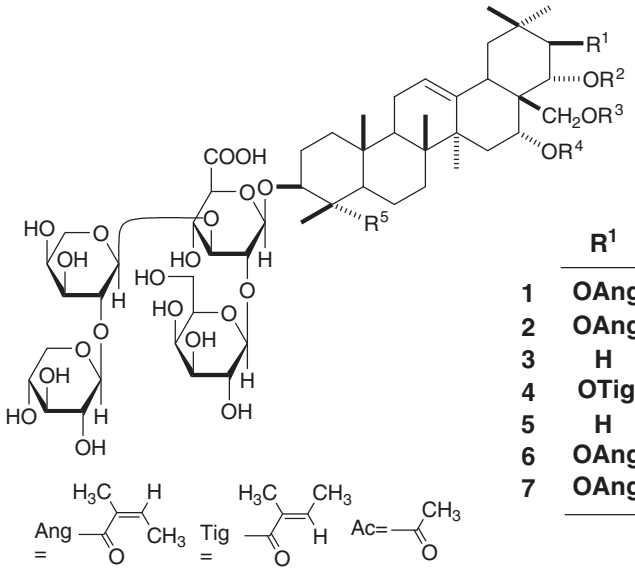

	R^1	R^2	R^3	R^4	R^5
1	OAng	Ac	H	H	CHO
2	OAng	H	Ac	H	CHO
3	H	Ang	H	H	CH_2OH
4	OTig	H	Ac	H	CHO
5	H	H	Ang	H	CHO
6	OAng	H	H	Ac	CH_2OH
7	OAng	H	Ac	Ac	CH_2OH

Fig. 1. Structures of compounds **1–7**.

Quinone reductase (QR), as a phase II enzyme, plays an important role in protecting cells against oxidatives by converting toxic quinones to hydroquinones and reducing oxidative cycling. It has been demonstrated that the induction of QR is an efficient strategy for reducing the risk of diseases related to exposure of toxins, mutagens and carcinogens. The QR can be used as a biomarker to evaluate the protection activity against tumor initiation of chemicals in the study on cancer chemoprevention [5,6]. In our investigation on the antitumor bioactive constituents of *C. sinensis*, the total tea seed saponin and the major triterpene saponin (theasaponin E1, mass content > 1.0%) exhibited QR inducing activity for hepa lclc7 cells (C = 20 μg/ml, IR = 2.9; C = 4 μg/ml, IR = 4.2 respectively). This is the first study to focus on the cytotoxicity and QR inducing activities of the tea seed saponins.

2. Experimental

2.1. Generals

Melting point was measured on a Yamaco-hot-stage and uncorrected. NMR spectra were recorded on a Bruker ARX-600 spectrometer, using TMS as an internal standard. ESI-MS was performed on a Finnigan LCQ mass spectrometer. Silica gel for chromatography was produced by Qingdao Ocean Chemical Group Co. of China. HPLC separations were performed on a Shim-pack PREP-ODS column (250 × 20 mm) equipped with a Shimadzu RID-6A refractive index detector and a Shimadzu LC-6AD series pumping system. The colorimetric assay was performed with a Microplate reader (EL × 800, BIO-TEK Instruments, Inc., USA).

2.2. Plant material

The seeds of *C. sinensis* were collected in Hanzhou, Zhejiang Province, China, in July, 2007. The plant material was identified by Prof. Qishi Sun (Shenyang Pharmaceutical University). A voucher specimen (no. 20070719) is deposited in the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

2.3. Extraction and isolation

2 kg dried seeds of *C. sinensis* was powdered and refluxed with hexane. The defatted seeds were further extracted with 75% EtOH for three times. Using our previously published method [5], the EtOH extract was dissolved in methanol and Et_2O was added to precipitate a crude tea seed saponin fraction (240.0 g, 12.0% from the dried seeds). Then the saponin fraction was subjected to HPD100 column chromatography by eluting with H_2O and MeOH to give a H_2O-eluted fraction (100.6 g) and a MeOH-eluted fraction (TTS, 125.0 g). The TTS fraction (30.0 g) was eluted with H_2O and MeOH by ODS column to give tea seed flavonoid glycosides (TF, 4.4 g) and tea seed triterpene saponin (TS, 22.7 g), respectively. Then, the TS fraction was purified by reversed-phase preparative HPLC with MeOH:1% aqueous AcOH (70:30, v/v) to afford eight fractions as Fr. 1 (0.9 g), Fr. 2 (0.32 g), Fr. 3 (0.30 g), Fr. 4 (5.55 g), Fr. 5 (3.81 g), Fr. 6 (1.89 g), Fr. 7 (5.76 g), and Fr. 8 (1.71 g). Fr. 4 to Fr. 8 were further purified by reversed-phase preparative HPLC with CH_3CN:1% aqueous AcOH or CH_3CN:MeOH:1% aqueous AcOH system. Fr. 4 was purified with CH_3CN:1% aqueous AcOH (40:60, v/v) to harvest theasaponin E_1 (**1**, 5.15 g, 1.07%). Fr. 5 was separated with CH_3CN:1% aqueous AcOH (40:60, v/v) to give theasaponin A_8 (**7**, 68.5 mg, 0.014%) and sub-fraction Fr. 5-8 (589 mg) which was further purified using CH_3CN:MeOH:1% aqueous AcOH (35:16:49, v/v/v) to give theasaponin C_1 (**3**, 135.1 mg, 0.028%). Fr. 6 was purified with CH_3CN:1% aqueous AcOH (43:57, v/v) to give assamsaponin C (**4**, 600.2 mg, 0.125%) and sub-fraction Fr. 6-5 (200 mg) which was also purified with CH_3CN:MeOH:1% aqueous AcOH (39:16:45, v/v/v) to give theasaponin A_9 (**6**, 76.5 mg, 0.016%).