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Hydrolysable Tannins and Related Compound having Cytotoxic Activity from the Fruits of *Terminalia chebula*

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The cytotoxicity-directed fractionation of MeOH extract of *Terminalia chebula* fruits led to the isolation of three hydrolyzed tannins and a related compound, gallic acid(I), 1,2,3,4,6-penta-O-galloyl-β-$_D$-glucopyranose(II), chebulagic acid(III) and chebulinic acid(IV), as active principles. They were shown to exhibit moderate cytotoxicity against cultured human tumor cell lines including A-549, SK-OV-3, SK-MEL-2, XF-498 and HCT-15 *in vitro*.

Key words: *Terminalia chebula*, Gallic acid, 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose, Chebulagic acid, Chebulinic acid, Cytotoxicity, Antitumor

INTRODUCTION

Tannins are widely distributed in the plant kindom and are classified on the basis of their structures into two groups, condensed and hydrolysable tannins. The latter is further classified into gallotannins having only galloyl groups and ellagitannins having hexahydroxydiphenoyl group(s), esterifying the polyalcohol core in the molecule. Several natural tannins and related compounds have been shown to have multiple biological activities. That is, inhibition of lipid peroxidation (Kimura *et al.*, 1984; Okuda *et al.*, 1983), decrease of mutagenicity of several mutagens (Okuda *et al.*, 1984), antiviral activity (Takechi *et al.*, 1985), decrease of blood urea-nitrogen content (Nishioka, 1983), various effects on lipolysis in fat cells (Kimura *et al.*, 1983), inhibitory effect on hyaluronidase (Lee *et al.*, 1993) and cytotoxic effect against some human tumor cell lines in vitro (Kashiwada *et al.*, 1992; Kashiwada *et al.*, 1993). The fruits of *Terminalia chebula* (Combretaceae) is one of the important folk medicines in Korea and China. It is known to be rich in tannins and has been widely used as an astringent and antidiarrheal agent. It has been suggested that most of the effects on these diseases are due to the tannins which are the main components in this plant. In the course of our continuing search for novel potent cytotoxic components in medicinal plants, the EtOAc soluble part of the fruits of *T. chebula* was found to show a significant inhibitory effect against human tumor cell lines including lungcarcinoma (A549), adenocarcinoma (SK-OV-3), malignant melanoma (SK-MEL-2), central nerve system tumor (XF498) and colon adenocarcinoma (HCT15). Subsequent cytotoxicity-directed fractionation led us to the isolation of known hydroly-sable tannins and related compounds as the active principles.

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MATERIALS AND METHODS

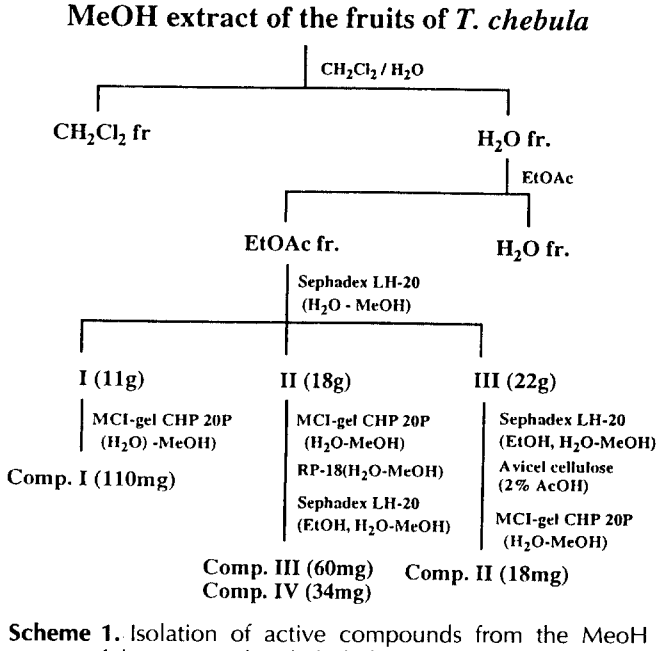
Melting points were determined on a Haake Buchler Melting point apparatus (U.K.) and are uncorrected. Optical rotations were measured with a JASCO DIP 140 digital polarimeter. ^{1}H-(300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on Bruker AM-300 spectrometers, with tetramethylsilane as an internal standard. FAB-MS were taken with a JEOL DX-303 instrument. Column chromatography was carried out with Sephadex LH-20 (25-100, Pharmacia Fine Chemical Industry Co. Ltd.), MCI-gel CHP 20P (75-150, Mitsubishi Chemical Industry Co. Ltd.), Avicel cellulose (Funacoshi) and Lichroprep RP-18 (40-63, Merck). Thin layer chromatography (TLC) was conducted on precoated silica-gel 60F_{254} plates and precoated cellulose F_{254} plates. Spots were visualized under UV illumination and by spraying 1% ethanolic $FeCl_3$ and 5% sulfuric acid.

Test for the cytotoxicity *in vitro*

Human tumor cell lines used in this experiment were obtained from National Cancer Institute (NCI) U.S.A., which were used in NCI as standard cell lines for the in vitro drug screening on antitumor activity. All experimental procedures followed the NCI's protocol, based on the SRB (sulforhodamine)-smear method (Skehan *et al.*, 1990). The maintenance of stock cell cultures and detailed experimental procedures were mentioned in a previous paper (Ryu *et al.*, 1992). Each of the test materials was dissolved in dimethylsulfoxide (DMSO) and then, diluted to the final concentration of DMSO to 0.5% with the medium.

Isolation of active compounds

The dried and powdered fruits (600 g) of *Terminalia chebula* purchased at market were extracted three times with MeOH for 4 hr under reflux. The MeOH solution was cooled, filtered and dried *in vacuo* to give a brown residue. The resultant MeOH extract was suspended in water, followed by the successive solvent partition with CH_2Cl_2, EtOAc, and which were tested for cytotoxicity *in vitro*. Active EtOAc soluble fraction was subjected to the Sephadex LH-20 column chromatography and eluted with H_2O containing increasing proportions of MeOH and afforded 3 fractions; I (11 g), II (18 g), III (22 g). Fraction I was chromatographed over MCI-gel CHP 20P (H_2O-MeOH) to give gallic acid (I, 110 mg). Fraction II was repeatedly chromatographed over MCI-gel CHP 20P (H_2O-MeOH), Lichroprep RP-18 (H_2O-MeOH) and Sephadex LH-20 (EtOH and/or H_2O-MeOH) to give chebulagic acid (III, 60 mg) and chebulinic acid (IV, 34 mg). Penta-O-galloyl-D-glucose (II, 18 mg) was obtained from fraction III by similar chromatographic separation (Scheme 1.).



Scheme 1. Isolation of active compounds from the MeOH extract of the *Terminalia chebula* fruits.

Gallic acid (I): colorless needles (H_2O), m.p. 270-272°C, $IR_{max}^{KBr}cm^{-1}$: 1650 (COO).

1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (II): a pale brown amorphous powder, $[\alpha]_D^{26}$+18.0° (c 0.8, acetone), ^{1}H-NMR (acetone-d_6+D_2O) δ: 5.66 (1H, t, J=8 Hz, H-2), 5.69 (1H, t, J=8 Hz, H-4), 6.05 (1H, t, J=8 Hz, H-3), 6.32 (1H, d, J=8 Hz, H-1), 7.00, 7.03, 7.08, 7.10, 7.16 (each 2H, s, galloyl H).

Chebulagic acid (III): a white powder, m.p. 240°C (decomp.), $[\alpha]_D^{21}$-50.6° (c 0.8, EtOH), ^{1}H-NMR (acetone-d_6+D_2O): 2.20 (2H, d, J=7 Hz, H-5'), 3.88 (1H, t, J=7 Hz, H-4'), 4.39 (1H, dd, J=12, 15 Hz, H-6), 4.68-4.84 (2H, m, H-5, 6), 4.92 (1H, d, J=7 Hz, H-2'), 5.12 (1H, dd, J=2, 7 Hz, H-3'), 5.23 (1H, d, J=4 Hz, H-4), 5.52 (1H, br s, H-2), 5.96 (1H, br s, H-3), 6.52 (1H, s, H-1), 6.65-7.05 (each 1H, s, HHDP-H), 7.13 (2H, s, galloyl H), 7.50 (1H, s, H-3).

Chebulinic acid (IV): colorless needles, m.p. 248-252°C (decomp.), $[\alpha]_D^{22}$+59.5° (c 1.0, MeOH), ^{1}H-NMR (acetone-d_6+D_2O): 2.28 (2H, d, J=7 Hz, H-5'), 3.96 (1H, t, J=7 Hz, H-4'), 4.73-4.81 (3H, m, H-5, 6), 4.97 (1H, dd, J=4, 7 Hz, H-2'), 5.10 (1H, dd, J=2, 3 Hz, H-4), 5.20 (1H, dd, J=1.5, 7 Hz, H-3'), 5.49 (1H, br s, H-2), 6.35 (1H, br s, H-3), 6.53 (1H, d, J=2 Hz, H-1), 7.07, 7.23, 7.27 (each 2H, s, galloyl H).

RESULTS AND DISCUSSION

Various medicinal plants containing tannins have been shown to be effective against cancers and tumors. The methanolic extract of the fruits of T. chebula yielded four kinds of active principles, according to the cytotoxicity-oriented fractionation monitering the inhibitory activity toward the proliferation of cultured human tumor cell lines. All of them were comprised of common hydrolyzed tannins and related compounds, which were identified as gallic acid(I), 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (II) (Lee *et al.*, 1990), chebulinic acid (III) and chebulagic acid (IV) (Yoshida *et al.*, 1982) by comparisons of their physical and spectral data with those of authentic samples. These components were found to exhibit moderate cytotoxic activity toward the human tumor cell lines including A-549, SK-OV-3, SK-MEL-2, XF498 and HCT15 (Table I). It was reported that hydrolysable tannins containing penta-O-galloyl glucopyranose and chebulagic acid showed potent cytotoxic activity against melanoma RPMI-7951 and the potency of activity in gallotannins was dependent

Table 1. The cytotoxic activity of compounds I - IV from the fruits of *Terminalia chebula*

Compound	ED_{50} (μg/ml)*				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
I	8.2	16.1	6.1	9.8	14.0
II	5.2	16.7	4.6	7.3	2.8
III	8.2	15.0	9.6	6.4	7.5
IV	7.5	8.7	5.7	4.3	3.6
Antimycin	0.48	5.50	0.27	0.39	0.41

*ED_{50} value was defined as a concentration (μg/ml) that caused 50% inhibition cell growth *in vitro*.

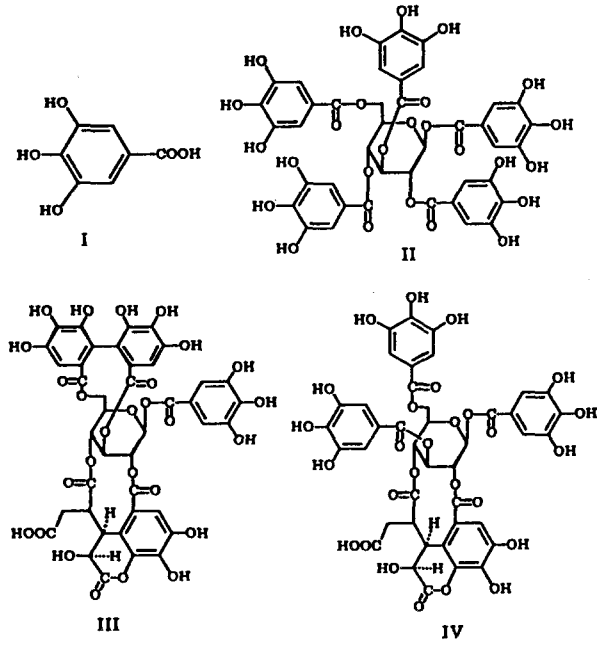


Chart 1. Cytotoxic compounds from the fruits of *Terminalia chebula*

on the polyalcohol in the molecule (Kashiwada *et al.*, 1992). In addition, penta-O-galloyl-$_D$-glucose, chebulagic acid and chebulinic acid were known to be a potent inhibitors of DNA topoisomerase II (Kashiwada *et al.*, 1993) *in vitro*.

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