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Phytochemical analysis and antileukemic activity of polyphenolic constituents of *Toona sinensis*

Akinori Kakumu [a], Masayuki Ninomiya [a,b], Mai Efdi [c], Morina Adfa [d], Masahiro Hayashi [e], Kaori Tanaka [e,f], Mamoru Koketsu [a,b,*]

[a] Department of Materials Science and Technology, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[b] Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[c] Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Andalas, 25163 Limau Manis, Padang, Indonesia
[d] Department of Chemistry, Faculty of Mathematics and Natural Science, Bengkulu University, Jalan Raya Kandang Limun, Bengkulu 38371, Indonesia
[e] Division of Anaerobe Research, Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan
[f] United Graduate School of Drug Discovery and Medicinal Information Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan

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ABSTRACT

Toona sinensis is a traditional Chinese medicine belonging to the *Meliaceae* family. The aim of this study was to identify the potential compounds responsible for anticancer activity of *T. sinensis*. The EtOAc extracts of leaves and woods of *T. sinensis* inhibited cell proliferation and induced apoptosis in human leukemia HL-60 cells. Our phytochemical research of these extracts led to the isolation of various polyphenolic constituents. The chemical structures were determined by spectroscopic analyses. Among isolates, gallic acid and loropetalin D showed inhibition of cell proliferation and possible induction of apoptosis in these cells. Overall, our results revealed the importance of *T. sinensis* as a chemopreventive medicinal plant. In addition, an analysis of structure–activity relationship indicated that the number of galloyl groups affects their antileukemic potency.

Plants and their chemical constituents play an important role as the source of innovative therapeutic agents for various conditions.[1–3] Traditional Chinese medicines have been widely applied for cancer care. Exploration of natural active compounds from medicinal plants for treatment of cancer has attracted substantial attention worldwide.

Toona sinensis is well known as a Chinese medicinal plant belonging to the *Meliaceae* family. It is widely distributed in China, Indonesia, Myanmar, Thailand, and Malaysia. The leaves of this plant have a long history of use in the folk medicine for the treatment of gastric ulcers, enteritis, dysentery, cerebrovascular, and cardiovascular diseases.[4–7] Its role in cancer therapy is also an active area of research. Chang et al., reported that the crude leaf extract possessed the antiproliferative effect and promoted apoptosis in human lung cancer A549 cells.[8] The extract was reported to show anticancer activity against several human cancer cells including human lung adenocarcinoma H441 cells,[9] human oral squamous carcinoma UM1, UM2, SCC-4, and SCC-9 cells,[10] human prostate cancer DU145 cells,[11] and human premyelocytic leukemia HL-60 cells.[12,13] They suggested that gallic acid isolated from the extract of leaves played a causative role of the biological activities, however, the detail investigation has not yet been carried out regarding anticancer compounds in *T. sinensis*. Within the scope of our ongoing program, aimed at the systematic chemical study of folk medicines with a biologically interesting profile, we investigated the chemical constituents of *T. sinensis*.

The leaves of *T. sinensis* were extracted at room temperature with *n*-hexane, EtOAc, and methanol in that order. Removal of solvents under reduced pressure afforded each extract (*n*-hexane; 120 g, EtOAc; 56 g, and methanol; 45 g from the leaves). The woods of *T. sinensis* were extracted with methanol at room temperature. The mixture was subsequently filtered and concentrated in vacuo to yield MeOH extract (94.6 g). This extract was suspended in water and partitioned successively with *n*-hexane, and EtOAc. Removal of solvents under reduced pressure afforded each extract (*n*-hexane; 7.1 g, EtOAc; 76.5 g, and water/residue 6.6 g from the woods). Initially, we evaluated the in vitro antileukemic activity of the extracts against human leukemia HL-60 cells using the CCK-8 assay.[14] Cells in the rapid phase of growth were exposed to tested extracts for 48 h. Treatment of the cells with each extract indicated that cell proliferation was inhibited by the EtOAc extracts of both leaves (TSL) and woods (TSW). At a final concentration of 100 μg/ml, these extracts exhibited the strong cytotoxicity, with

* Corresponding author. Tel./fax: +81 58 293 2619.
E-mail address: koketsu@gifu-u.ac.jp (M. Koketsu).

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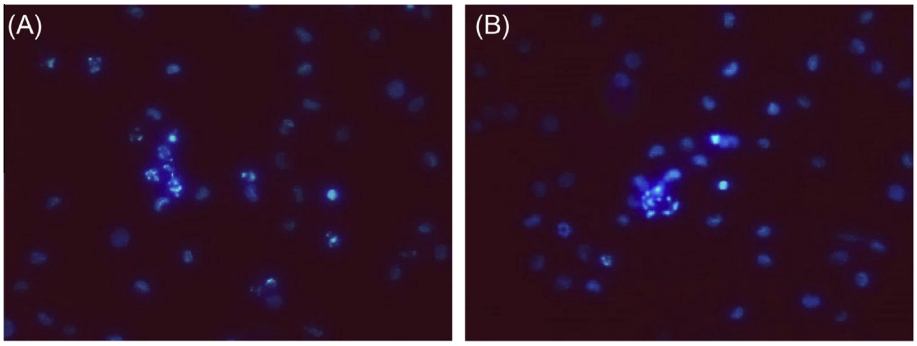

Figure 1. Morphological changes of HL-60 cells induced by leaf extract (TSL, A) and wood extract (TSW, B) of *Toona sinensis* at a final concentration of 100 μg/ml. Cells were treated with the extracts for 48 h, and were stained with Hoechst 33342.

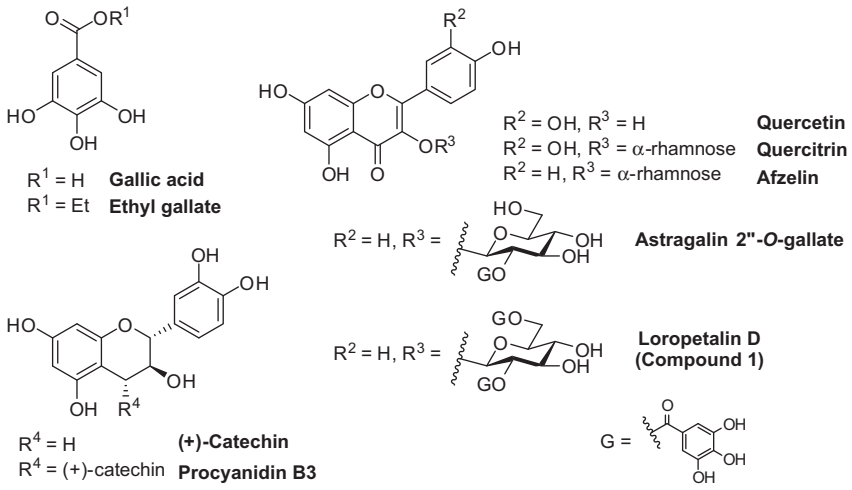

Figure 2. Chemical structures of isolated polyphenols.

Table 1
Cytotoxicity of isolated polyphenols in human leukemia HL-60 cells (means ± SEMs, $n = 6$)

Compound	Cell viability (% of control)	Compound	Cell viability (% of control)
Gallic acid	4.3 ± 0.9	Astragalin 2″-O-gallate	48.3 ± 5.6
Ethyl gallate	55.7 ± 8.4	Loropetalin D	24.6 ± 3.1
Quercetin	29.7 ± 3.6	(+)-Catechin	47.0 ± 8.7
Quercitrin	46.8 ± 3.9	Procyanidin B3	58.9 ± 2.6
Afzelin	45.8 ± 6.7		

cell survival rates of 12.0% (TSL) and 52.8% (TSW), respectively. Furthermore, we observed morphological changes in the cells stained with Hoechst 33342.[15] These extracts caused fragmentation of the nuclei and condensation of the chromatin, both of which are signs of apoptosis (Fig. 1).

To identify the potential compounds responsible for these properties, the EtOAc extracts of leaves (TSL) and woods (TSW) of *T. sinensis* were separated by column chromatography (CC) using silica gel and Sephadex LH-20, and RP-HPLC. Through several processes, a variety of polyphenolic constituents were isolated. Seven compounds were isolated from the extract of leaves. Compound **1**

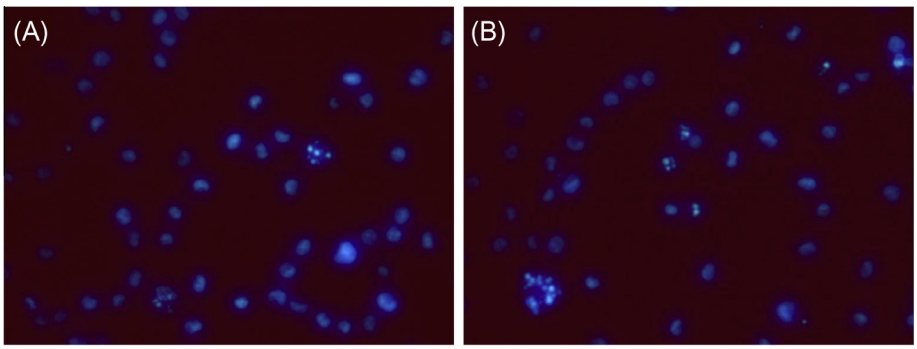

Figure 3. Morphological changes of HL-60 cells induced by gallic acid (A) and loropetalin D (B) at a final concentration of 50 μM. The cells were stained with Hoechst 33342.