Hydrolysable Tannins and Related Compound having Cytotoxic Activity of Geranium Wilfordii Maxim

1, 2Ping Li, 1Luoshao Zhao, 1Youyou Du, 2Yinman Feng and 2Yanhong Li
1Department of Cardiology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
2Academician Expert Workstation of Zhengzhou Health Department, Zhengzhou, China

Abstract: In order to exhibit moderate cytotoxic activity toward the human tumor cell lines including A549, SK-OV-3, HT-1080, K562 and S180, the cytotoxicity-directed fractionation of MeOH extract of Geranium wilfordii Maxim 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 A549, SK-OV-3, HT-1080, K562 and S180 in vitro.

Keywords: Antitumor, cytotoxicity, gallic acid, geranium wilfordii maxim

INTRODUCTION

Geranium wilfordii Maxim is native to China and is widely distributed in China. In Traditional Chinese Medicine, Geranium wilfordii Maxim is one of the important folk medicines. It is used as rheumatism and dredging meridians as well as to arrest diarrhea and treat dysentery. Geranium wilfordii Maxim has been reported to exhibit a variety of biological activities including anticancer (Yang et al., 2010; Wang et al., 2004) and mutagenic (Okuda et al., 1983) antibacterial (Serkedj, 1997) Anti-Oxidant, protecting liver and ant-HIV (Lei et al., 2008). It is known to be rich in tannins and has been widely used as an astringent and an ant diarehal agent. Furthermore, several flavonoids, organic acids, tannins and phenolic compounds have been isolated from Geranium wilfordii Maxim (Lei et al., 2008; Du et al., 2003; Mavlyanov et al., 1997). 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 Geranium wilfordii Maxim was found to show a significant inhibitory effect against human tumor cell lines including lung carcinoma (A549) adenocarcinoma (SK-OV-3), Human fibro sarcoma cells (HT-1080), Human leukemia cell (K562) and sarcoma (S180). Subsequent cytotoxicity-directed fractionation led us to the isolation of known hydrolysable tannins and related compounds as the active principles.

In the past, giant forging press are used in the active beams under the four corners of the layout of a passive synchronous control systems, which will not only increase the cost of investment in equipment and its maintenance, but also some special hydraulic press due to space issues, cannot be arranged passive synchronization control systems.

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. 1H(300 MHz) and 13C(NMR (75 MHz) spectra were recorded on Bruker AM-300 spectrometers, with tetra methyl’s lane 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, Shanghai Sino harm Chemical Reagent Co., Ltd.), MCI-gel CHP 20P (75-150, Shanghai Sino harm Chemical Reagent Co., Ltd.) Thin Layer Chromatography (TLC) was conducted on precoated silica-gel 60F254 plates and precoated cellulose F254 plates. Spots were visualized under UV illumination and by spraying 1% ethanolic FeCl3 and 5% sulfuric acid.

Test for the cytotoxicity in vitro: Human tumor cell lines used in this experiment were obtained from Shanghai Institutes for Biological Sciences (SIBS) which were used in SIBS as standard cell lines for the in vitro drug screening on antitumor activity. All experimental procedures followed the SIBS's protocol.
Adv. J. Food Sci. Technol., 5(3): 255-257, 2013

Fig. 1: Isolation of active compounds from the MeOH extract of the geranium wilfordii maxim based on the SRB (Sulforhodamine) smear method (Skehan et al., 1990). Each of the test materials was dissolved in Di Methyl Sulf Oxide (DMSO) and then, diluted to the final concentration of DMSO to 0.5% with the medium.

ISOLATION OF ACTIVE COMPOUNDS

The dried and powdered Geranium wilfordii Maxim (800 g) purchased at market were extracted three times with MeOH for 4 h 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 CH₂Cl₂, EtOAc and which were tested for cytotoxicity in vitro. Active EtOAc soluble fraction was subjected to the Sephadex LH20 column chromatography and eluted with H₂O containing increasing proportions of MeOH and afforded 3 fractions; I (18 g), II (24 g), III (29 g). Fraction I was chromatographic over MCI-gel CHP 20P (H₂O:MeOH) to give Gallic acid (I, 190 mg). Fraction II was repeatedly chromatographic over MCI-gel CHP 20P (H₂O:MeOH), Lichroprep RP-18 (H₂O:MeOH) and Sephadex LH-20 (EtOH and/or H₂O:MeOH) to give chebulagic acid (III, 80 mg) and chebulinic acid (IV, 38 mg). Penta-O-galloyl-β-D-glucose (II, 25 mg) was obtained from fraction III by similar chromatographic separation (Fig. 1).

Gallic acid (I): Colorless needles (H₂O), m.p. 270-272°C, IR max ν/cm: 1650 (COO).

1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucopyranose (II): Apale brown amorphous powder, [α]D 50.6° (c 0.8, acetone), 1H-NMR (acetone-d₆+D₂O) δ: 5.66 (1 H, t, J = 8 Hz, H-2), 5.69 (1 H, t, J = 8 Hz, H-4), 6.05 (1 H, t, J = 8 Hz, H-3), 6.32 (1 H, d, J = 8 Hz, H-1), 7.00, 7.03, 7.08, 7.10, 7.16 (each 2 H, s, galloyl H).

Chebulagic acid (III): A white powder, m.p. 240°C (decomp.), [α]D 50.6° (c 0.8, EtOH), 1H-NMR (acetone-d₆+D₂O) δ: 2.20 (2 H, d, J = 8 Hz), 3.88 (1 H, t, J = 7 Hz, H-3), 4.39 (1 H, dd, J = 12, 15 Hz), 4.68-4.84 (2 H, m, H-5, 6), 4.92 (1 H, d, J = 7 Hz, HZ²), 5.12 (1 H, dd, J = 2, 7 Hz, H-3'), 5.23 (1 H, d, J = 4 Hz, H-4), 5.52 (1 H, br s, H-2), 5.96 (1 H, br s, H-3), 6.52 (1 H, s, H-1), 6.65-7.05 (each 1 H, s, HHDP₂H), 7.13 (2 H, s, galloyl H), 7.50 (1 H, s, H-3).

Chebulinic acid (IV): colorless needles, m.p. 248-252°C (decomp.), [α]D 50.6° (c 1.0, MeOH) 1H-NMR, (acetone-d₆+D₂O): 2.28 (2 H, d, J = 7 Hz), 3.85 (1 H, t, J = 7 Hz, H-3), 4.73-4.81 (3 H, m, H-5, 6), 4.97 (1 H, dd, J = 4, 7 Hz, H-2), 5.10 (1 H, dd, J = 2, 3 Hz, H-4), 5.20 (1 H, dd, J = 1.5, 7 Hz, H-3'), 5.49 (1 H, br s, H-2), 6.35 (1 H, br s, H-3), 6.53 (1 H, d, J = 2 Hz, H-1), 7.07, 7.23, 7.27 (each 2 H, 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 Geranium wilfordii Maxim. Four kinds of active principles, according to the cytotoxicity oriented fractionation monitoring 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) chebulinic acid (III) and chebulagic acid (IV). 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 A549, SK-OV-3, HT-1080, K562 and S180 (Table 1).

It was reported that hydrolysable tannins containing penta-O-galloyl glucopyranoside and chebulagic acid showed potent cytotoxic activity against melanoma RPMI-7951 and the potency of

| Compound | A549 | SK-OV-3 | HT-1080 | K562 | S180 |
|----------|------|---------|---------|------|------|
| I        | 8.50 | 16.7    | 6.40    | 9.70 | 14.9 |
| II       | 5.40 | 16.9    | 4.70    | 7.20 | 2.90 |
| III      | 8.50 | 15.2    | 9.80    | 6.30 | 7.80 |
| IV       | 7.80 | 8.90    | 5.90    | 4.20 | 3.90 |
| Antimycin| 0.49 | 5.58    | 0.29    | 0.38 | 0.45 |

*: ED₅₀ value was defined as a concentration (µg/mL) that caused 50% inhibition cell growth in vitro
activity in gallotannins was dependent on the polyalcohol in the molecule (Khiqada, 1992). In addition, penta-O-galloyl-β-D-glucose, chebulagic acid and chebulinic acid were known to be potent inhibitors of DNA topoisomerase II (Zhang et al., 2009; Takashi et al., 1999) in vitro.

ACKNOWLEDGMENT

The study was financially supported by tackle key problems in science and technology Project Funding of Zhengzhou and Zhengzhou Health School (code 121PPTGG487). The authors are also grateful to prof. L.d. Wang and Dr. g.w. Yin for their contribution. Luosha Zhao is a corresponding author for this study.

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