

Research Article

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

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Abstract: In order to exhibit moderate cytotoxic activity toward the human tumor cell lines including A-549, SK-OV-3, HT-1080, K562 and S180, the cytotoxicity-directed fractionation of Me OH 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) ant mutagenic (Okuda *et al.*, 1983) antibacterial (Serkedj, 1997) Anti-Oxidant, protecting liver and anti-HIV (Lei *et al.*, 2008). It is known to be rich in tannins and has been widely used as an astringent and antidiarrheal 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.

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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,

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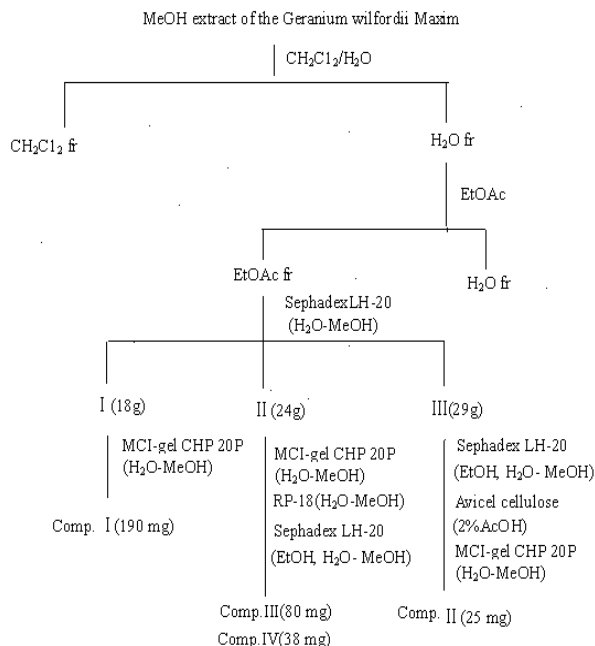


Fig. 1: Isolation of active compounds from the MeOH extract of the geranium wilfordii maxim

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 vacuous 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 (18 g), II (24 g), III (29 g). Fraction I was chromatographic over MCI-gel CHP 20P (H_2O -MeOH) to give Gallic acid (I, 190 mg). Fraction II was repeatedly chromatographic 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, 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 (H_2O), m.p. 270-272°C, IRmax^{KBr}/cm: 1650 (COO).

1, 2, 3, 4, 6-penta-O-galloyl- β -D-glucopyranose (II): Apale brown amorphous powder, $[\alpha]_D^{26} +18.0$ (c 0.8, acetone), ¹H-NMR (acetone- $\text{d}_6 + \text{D}_2\text{O}$) 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 power, m.p. 240°C (decomp.), $[\alpha]_D^{21} 50.6^\circ$ (c0.8, EtOH), ¹H-NMR (acetone- $\text{d}_6 + \text{D}_2\text{O}$): 2.20 (2 H, d, J = 7 Hz, H-5'), 3.88 (1 H, t, J = 7 Hz, H-4'), 4.39 (1 H, dd, J = 12, 15 Hz, H6), 4.68-4.84 (2 H, m, H-5, 6), 4.92 (1 H, d, J = 7 Hz, H2'), 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, HHDH-H), 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.), $[\alpha]_D^{22} +59.5^\circ$ (c1.0, Me OH) ¹H-NMR, (ac-eton-d₆+D₂O): 2.28 (2 H, d, J = 7 Hz, H-5'), 3.96 (1 H, t, J = 7 Hz, H-4'), 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

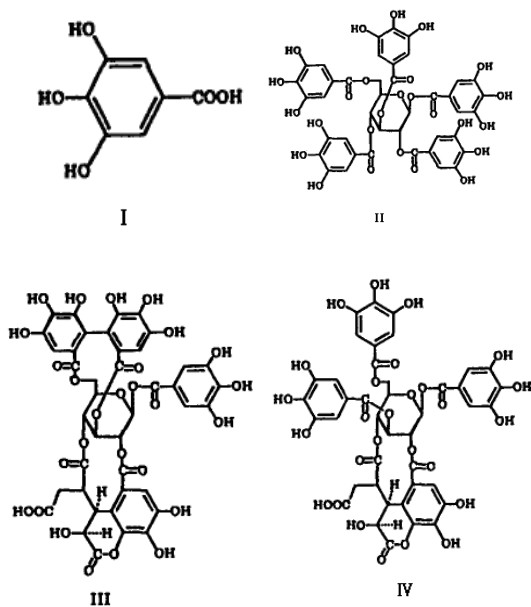
Various medicinal plants containing tannins have been shown to be effective against cancers and tumors. The methanolic extract of the *Geranium wilfordii* Maxim. Four kinds of active principles, according to the cytotoxicity-oriented fractionation monitoring the inhibitory activity toward the proliferation 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 A-549, SK-OV-3, HT-1080, K562 and S180 (Table 1).

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

Table 1: The cytotoxic activity of compounds I-IV from geranium wilfordii maxim

Compound	ED ₅₀ (µg/mL)*				
	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*.

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REFERENCES

Du, S., Y. Zhou and L. Wei. 2003. Studies on chemical constituents of *Geranium eristemon* [J]. Chinese Pharmaceut. J., 34(6): 501-502.
 Khiqada, Y., 1992. Antitumor agents 129 tannis and related compounds as selective cytotoxic agents [J]. J. Nat. Prod., 55(8): 1033-1043.

Lei, H., B. Wei, L. Qiang, B. Dong and W. Luxue 2008. Chemical constituents of erodium stephanianum Willd [J]. Northwest Pharmaceut. J., 23(1): 18-19.
 Mavlyanov, S.M., S.Y. Mambekov and F.G. Karnayev, 1997. Tannins of gerqniuno sanguinetma [J]. Chern. Natural Compounds, 33(2): 179.
 Okuda, T., Y. Kimura, H. Okuda, T. Yoshida, T. Hatano and S. Arichi, 1983. Studies on the activities of tannins and related compounds from medicinal plants and drugs I: Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. Chem. Pharm. Bull., 31: 1625.
 Serkedj, J., 1997. Antinfective activity of a plant preparation form geranium sanguineuml. Pharmaize, 52(10): 799.
 Skehan, P., R. Streng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney and M.R. Boyd, 1990. New colorimetric cytotoxicity assay for anticancer-drug screening [J]. Natl. Cancer. Inst., 82: 1107-1112.
 Takashi, M., Z. Cun, A. Kuniaki, O. Hidehumi, K. Tadash, 1999. Antitumor and hypoglycemic activities of polysaccharides from the sclerotia and mycelia of *Inonotus obliquus* (Pers.: Fr.) Pil. (Aphyllphoromycetidae) [J]. Int. J. Med. Mush., 1(1): 301-316.
 Wang, X.H., K.F. Bastow, C.M. Sun, L. Yun-Lian, Y. Hsi-Jung, D. Ming-Jaw, W. Tian-Shung, N. Seikou and L. Kuo-Hsiung, 2004. Antitumor agents 239 isolation structure elucidation total synthesis and anti-breast cancer activity of neotanshinlactone from *Salvia miltiorrhiza*. J. Med. Chem, 47: 5816-5819.
 Yang, Y.C., L. Ji, Z. YuanGang, F. Yu-Jie, L. Meng, W. Nan and L. Xiao-Lei, 2010. Optimisation of microwave assisted enzymatic extraction of corilagin and geraniin from geranium sibiricum linne and evaluation of antioxidant activity. Food Chem., 122(1): 373-380.
 Zhang, J., L. Li, K. Sung-Hoon, A.E. Hagerman and L. Junxuan, 2009. Anti-cancer, anti-diabetic and other pharmacologic and biological activities of penta-galloyl-glucose [J]. Pharmaceut. Res., 26(9): 2066-2080.