머루나무 가지에서 분리한 stilbene 성분의 세포독성 효과 충남대학교: 도티하, 진전성, 윤의중, 이익수, <u>배기환</u>*

Stilbene Constituents and Their Cytotoxicity Inhibitory Property From The Stems of Vitis amurensis Rupr.

College of pharmacy, Chungnam National University

Do Thi Ha, Quan Cheng Chen, Ui Jung Youn, Ik soo Lee, and

KiHwan Bae*

Objectives:

Vitis species are a convenient alimentary source of salutary phytochemicals. Recently, reported papers are only focused on natural occurrence, extraction methods, bioavailability, analytical detection, and metabolism of resveratrol, as well as its effects on cancer, anti-inflammatory, atherosclerosis, and neutron not on its analogs such as resveratrol polymers (resveratrol dimers, trimers, and tetramers). To date, the compositions of the roots have been investigated sufficient detail, but the stems have been rarely studied both constituents and bioactivities. Therefore, in this study, we focused on the extraction, identification of isolates, quantity analysis stilbene compounds presenting in the stems, and their cytotoxicity inhibitory evaluation.

Materials and Methods

Materials

The stems of V. amurensis were collected in Daejeon, Korea, in July 2005.

Methods:

The MeOH extract of the stems were successively partitioned with hexane, EtOAc, and BuOH solvents to give hexane-, EtOAc-, and BuOH-soluble fractions, respectively, and water fraction. A bioassay-guided extraction recommended that the EtOAc-soluble fraction exhibited significant cytotoxicity inhibitory property against cultured L1210 and K562 cancer cell lines. Repeated chromatography of this fraction using several columns with different packing material such as silica gel, bonded phase slica gel (C18), sephadex LH-20) to isolate stilbene compounds. Cytotoxicity assay was performed following the method of Mosmann.

Results:

Seven stilbene compounds named gnetin H (1), 2-r-viniferin (2), amurensin G (3), resveratrol (4), ampelopsin F (5), (-)-Ampelopsin A (6), and piceid (8), together with one tannin compound named (+)-catechin (7) were obtained from the *Vitis amurensis* stems. Among those, comp. 1–3 have been firstly isolated from the stem. Their structures have been identified on the basis of their physicochemical and spectroscopic analyses. Further more, these compounds were quantified by mean of HPLC system coupled with UV detector. Concentration of six isolates (2–6) in the EtOAc extract were evaluated 5.0 %, 1.27 %, 8.81 %, 3.71 %, 0.76 %, 1.67 %, respectively. Evaluation the cytotoxicity inhibition of seven stilbene compounds resulted that comp. 1 possessed a good activity with the IC50 value of 3.59 μ g/mL. Comp. 5–6 showed the moderate activity only with the IC50 value of 27.3 μ g/mL, and other ones, comp. 3, 10 exhibited no activity at the concentration over 30 μ g/mL.

^{*}주저자연락처(Corresponding author): KiHwan Bae E-mail: baekh@cnu.ac.kr Tel. 042-821-5925

Fig. 1. All isolates (1-7) from the stems of *Vitis amurensis*

Table 1. Cytotoxicity of compounds against cultured L1210 and K562 cancer cell lines

No.	Compound	IC ₅₀ a) (g/ml)				
	S	L121	0	K5	62	
1	1	27.3	3.2		>3	0
2	2	>30			>3	0
3	3	13.3	1.9		18.93	0.37
4	4	3.59	0.50		7.07	0.41
5	5	14.4			18.14	0.35
6	6	>30 b)			>30	
7	8	11.2	1.1		9.61	0.30
8	Adriamycin ^c	1.28	0.13			

 IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number and, the results are means tandard deviation of three independent replicates. $^{\rm c}$ The IC_{50} greater than 30 g/ml was considered to be no cytotoxicity. Positive control substance

Table 2. Content of isolated compounds from EtOAc fraction of the *Vitis* amurensis stems.

Compounds	Content (mg/100 mg EA Fr., $n = 3$)
1	5.01
3	1.27
4	8.81
5	3.71
6	0.76
8	1.67