ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jul; 21 (7): 633 - 636
http://www.chinaphar.com

Antioxidant activity of polyphenols from seeds of *Vitis amurensis in vitro*

WANG Jian-Nong[1], CHEN Ying-Jie[2], HANO Yoshio[3], NOMURA Taro[3], TAN Ren-Xiang (*State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, China*; [2]*Department of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang 110015, China*; [3]*Faculty of Pharmaceutical Science, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan*)

KEY WORDS *Vitis amurensis*; *Vitaceae*; antioxidants; catechin; procyanidin B_2; procyanidin B_5; procyanidin B_5 3′-O-gallate; amurensisin

ABSTRACT

AIM: To study the antioxidant action of five polyphenols (+) catechin, procyanidin B_2, procyanidin B_5, procyanidin B_5 3′-O-gallate, and amurensisin isolated from the seeds of *Vitis amurensis*. **METHODS**: The mouse liver homogenate lipid peroxidation assay was applied for the evaluation of the antioxidant activity *in vitro*. **RESULTS**: (+) Catechin, procyanidin B_2, procyanidin B_5, procyanidin B_5 3′-O-gallate, and amurensisin showed antioxidant activity with the IC_{50} values of 0.47, 0.25, 0.10, 0.02, and 0.03 mmol/L, respectively. The IC_{50} value of vitamin E used as a positive control was 0.13 mmol/L. The structural activity relationship was also analyzed. Procyanidins carrying a galloyl group possessed higher anti-lipid peroxidation activities. All dimers were found to be more potent than the non-galloylated momomer such as (+) catechin. However, the activity of the 4→6 linked dimer seemed more preferable than 4→8 linked dimer. **CONCLUSION**: Procyanidin B_5, procyanidin B_5 3′-O-gallate, and amurensisin showed a more antioxidant activity than vitamin E did, and their activity is dependent on their substitution and polymerization patterns.

INTRODUCTION

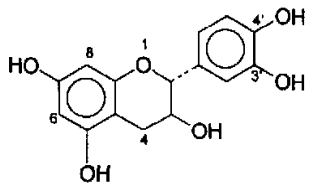
The oxygen-derived species such as the superoxide and hydroxyl radicals play an important role in tissue damage involved in many pathological processes including aging, inflammation, cancer, atherosclerosis, and reoxygenation of ischemic tissue[1-4]. Some antioxidant natural polyphenols such as flavonoids (in particular those derived from edible plants) have been shown to be applicable in chemoprevention[5,6]. However, the studies on the antioxidant effect of the purified procyanidins has been quite limited to date, presumably owing to the difficulties in the purification of these products[3]. Sometimes, the antioxidant action of procyanidins had to be evaluated in the mixture[4]. *Vitis amurensis* Rupr (Family: *Vitaceae*), a vine plant distributed mainly in Northern area of China, is a well known starting material for wine production. A large quantity of grape seeds are mainly discarded as a kind of "by-product" from the wine-making industry, or used for cattle breeding in very limited areas. However, grape seeds have been utilized as a tonic folk remedy in traditional Chinese medical practice. Early in 1969, *V vinifera* was disclosed to be a rich source of procyanidins, 60 % of which were present in the seed[7]. As a continuation of this finding, we recently characterized three known [(+) catechin (1), procyanidin B_2(2), and procyanidin B_5(3)] and two new polyphenols procyanidin [B_5 3′-O-gallate (4) and amurensisin (5)] from the seeds of *V Amurensis*[8]. Compounds 1 - 5 are representatives of five different types of polyphenols. Previously, compounds 1 - 3 have been reported to be oxygen free radical scavengers[3]. This observation prompted us to reisolate these products from the seeds of *V amurensis*, and to evaluate their antioxidant action using a mouse liver homogenate lipid peroxidation assay to obtain a better understanding of the relationship between their structure and the antioxiant activity.

MATERIALS AND METHODS

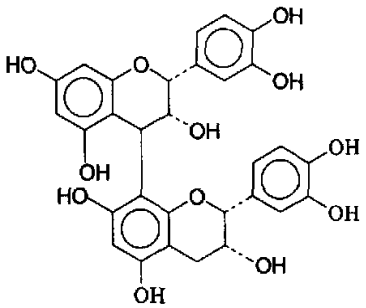
Plant material Seeds of *V amurensis* were collected in October 1998 from the suburbs of Tonghua City,

[1] Correspondence to Dr WANG Jian-Nong.
Phn 86-25-359-3201. Fax 86-25-359-3201.
Received 1999-09-06 Accepted 1999-12-27

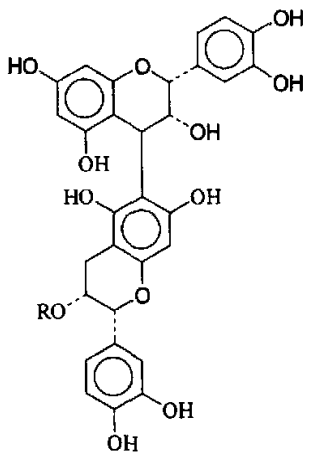
ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jul; 21 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629



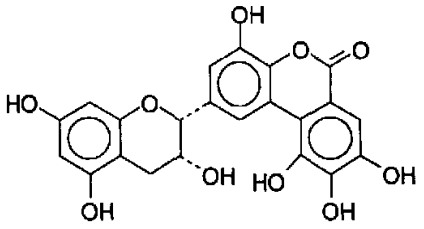
(+)-catechin (**1**)



procyanidin B_2 (**2**)



procyanidin B_5 (**3**) R = H
procyanidin B_5 3'-O-gallate (**4**) R = galloyl



amurensisin (**5**)

Jilin Province, China. The voucher specimen (SY96031), identified by Prof ZR Jiang, was deposited in the Herbarium of Shenyang Pharmaceutical University (Shenyang, China).

Animal and chemicals Kunming male mice (weighing 25 – 28 g) were supplied by the Experimental Animal Center of China Pharmaceutical University. All the chemicals and solvents used in the study were of analytical grade.

Fractionation The air-dried seeds of *V amurensis* (8 kg) were coarsely powdered and then extracted twice (for 24 h each) with 80 % ethanol (8 L) at room temperature. Evaporation of ethanol *in vacuo* from the extract gave a suspension which was partitioned successively with chloroform, ethyl acetate and *n*-butanol affording chloroform (C, 10 g), ethyl acetate (E, 101 g) and *n*-butanol soluble parts (B, 232 g), respectively. The ethyl acetate fraction (fraction E) was subjected to Diaion HP-20 column eluted with water containing gradually increasing amounts of methanol to obtain two fractions (Fr 1 – 2). Gel filtration of Fr 1 on Sephadex LH-20 eluted with ethanol followed by recrystallization in methanol afforded compound 1 (2.3 g), and the mother liquor was further separated by Sephadex LH-20 using aqueous ethanol as the flowing phase to give compounds 2 (48 mg), 3 (55 mg). Successive chromatography of Fr 2 over Sephadex LH-20, Toyopearl HW-40, and MCI gel CHP20P eluted with mixture of methanol-water (3:7) afforded compounds 4 (35 mg) and 5 (25 mg). The more detailed isolation of the polyphenols (1 – 5) from fraction E was nearly the same as that established previously[8].

Bioassay The bioassay was carried out as described elsewhere[9]. Liver was taken from Kunming male mice to make liver homogenate (5 %) with saline. The liver homogenate (1.5 mL) was transferred to a test tube, to which 0.2 mL of ascorbic acid (AA, 6 mmol/L), 0.1 mL of $FeCl_2$ (4 mmol/L), and 0.1 mL of polyphenol solution in water were added. Equal amounts of vitamin E (0.5 mmol/L) in EtOH and 0.2 mL of the AA liquor (mixed with 0.1 mL of the $FeCl_2$ solution) were applied as positive and negative controls, respectively. Each sample was tested at five concentrations (Tab 1). After incubating the mixture in a vibratory incubator at 37 ℃ for 1.5 h, 1.5 mL of trifluoacetic acid (20 %) was added (a blank control tube was treated equally for zeroing the spectrometer). This was immediately followed by centrifugation (700 × *g*) for 10 min. The supernatant (1.5 mL) was transfered to another tube, to

Tab 1. Effects of the compounds 1 – 5 on the formation of TBA-RS induced by $FeCl_2$-AA in a mouse liver homogenate *in vitro*. $n = 5$. $^cP < 0.01$ *vs* $FeCl_2$-AA treated control (negative control). $^fP < 0.01$ *vs* Vitamin E (positive control).

Compound	Conc/ mmol·L^{-1}	TBA-RS/ nmol MDA·g^{-1} (wet tissue)	Inhibition/%	IC_{50}/mmol·L^{-1}
$FeCl_2$-AA Treated Control		266 ± 9		
(+)Catechin (1)	0.055	254 ± 7	4.5	0.470 ± 0.010
	0.136	239 ± 5^c	10.1	
	0.275	205 ± 12^c	22.7	
	0.412	159 ± 10^c	40.0	
	0.550	111 ± 7^c	58.1	
Procyanidin B_2(2)	0.055	239 ± 9^c	10.0	0.250 ± 0.020
	0.136	196 ± 9^c	26.1	
	0.275	114 ± 5^c	57.1	
	0.412	42 ± 5^c	84.0	
Procyanidin B_5(3)	0.013	242 ± 13	9.0	0.100 ± 0.010^f
	0.033	224 ± 11^c	15.8	
	0.065	181 ± 10^c	32.0	
	0.097	153 ± 9^c	42.5	
	0.130	108 ± 6^c	56.0	
Procyanidin B_5 3'-O-gallate (4)	0.008	214 ± 9^c	19.5	0.0200 ± 0.0010^f
	0.021	139 ± 7^c	47.6	
	0.041	29 ± 5^c	89.0	
	0.061	5 ± 2^c	98.2	
Amurensisin (5)	0.007	228 ± 12^c	14.2	0.0300 ± 0.0010^f
	0.017	174 ± 8^c	34.5	
	0.034	72 ± 5^c	73.0	
Vitamin E	0.050	225 ± 10^c	15.2	0.1300 ± 0.0010
	0.100	185 ± 8^c	30.5	
	0.200	94 ± 8^c	64.8	

which 1.0 ml of thiobarbituric acid (6.7 g/L) was added, followed by heating on a boiling water bath for 10 min. After cooling down, the absorption of the solution was determined at 532 nm on an UV-Visible spectrometer.

Statistical analysis The amount of malonaldehyde (MDA) that formed was calculated based on the standard, the tetraethoxy propane, and the inhibition rate was obtained by referring to control groups, followed by statistical analysis with *t*-test.

RESULTS

As summarized in Tab 1, the five tested polyphenols showed antioxidant activities on the mouse liver homogenate lipid peroxidation assay, and the action was dose-dependent (Fig 1). Compounds 3 – 5 (IC_{50}: 0.10, 0.02, and 0.03 mmol/L, respectively) possessed stronger antioxidant activities than the positive control vitamin E (IC_{50}: 0.13 mmol/L).

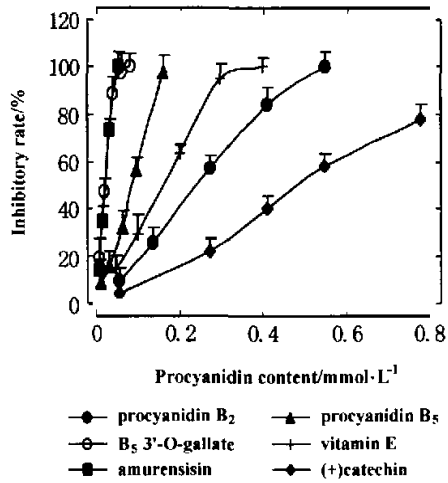


Fig 1. The inhibitory effect on MDA of the polyphenols isolated from seeds of *V amurensis*.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jul; 21 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

DISCUSSION

Concerning the relationship between the structure and the antioxidant activity, all dimers (2 – 4) were found to be more antioxidative than the momomer (+)-catechin (1), and the newly characterized galloylated dimer (4) and monomer (5) were more antioxidative than the known ones (1 – 3) which bear no galloyl group. Comparison between the antioxidant activities of procyanidins 3 and 4 showed that galloylation at 3α-hydroxy group of the former could multiply its antioxidant activity. Furthermore, the antioxidant activity of 5 was fifteen times stronger than that of 1 (IC_{50}: 0.47 mmol/L). These observations demonstrated that galloylation of procyanidins can increase substantially the magnitude of their antioxidant action. On the other hand, comparison between the antioxidant activities of isomeric procyanidins 2 (IC_{50}: 0.25 mmol/L) and 3 showed that the 4→6 linkage seems more preferable than the 4→8 dimerization. This finding was comparable to the difference in oxygen free radicals trapping capability between the 4→6 and 4→8 linked dimers[3].

Present investigation demonstrated that three of the five phytochemically characterized polyphenols showed more antioxidant activities than vitamin E. As all the compounds are abundant in the seeds of the edible plant *V amurensis*, the toxicity can be negligible if they would be developed into antioxidants to be used as drug and/or food additives.

ACKNOWLEDGEMENT This work was financed in part by grants for RXT from NNSF (№ 39725033 and 39670873). Dr JN Wang is grateful to the educational promoting program of Japan for a visiting fellowship to Toho University, Japan.

REFERENCES

1 Wang H, Nair MG, Strasburg GM, Booren AM, Gray JI. Novel antioxidant compounds from tart cherries (Prunus cerasus). J Nat Prod 1999; 62: 86 – 8.
2 Marx JL. Oxygen free radicals linked to many diseases. Science 1987; 235: 529 – 31.
3 Ricardo-da-Silva JM, Darmon N, Fernandez Y, Mitjavila S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. J Agric Food Chem 1991; 39: 1549 – 52.
4 Bos MA, Vennat B, Meunier MT, Pouget MP, Pourrat A, Fialip J. Procyanidins from tormentil: antioxidant properties towards lipoperoxidation and anti-elastase activity. Biol Pharm Bull 1996; 19: 146 – 8.
5 Zhao BL, Li XJ, He RG, Cheng SJ, Xin WJ. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. Cell Biophys 1989; 14: 175 – 85.
6 Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 1988; 37: 837 – 41.
7 Tsai Su C, Singleton VL. Identificastion of three flavan-3-ols from grapes. Phytochemistry 1969; 8: 1553 – 58.
8 Wang JN, Hano Y, Nomura T, Chen YJ. Procyanidins from the seeds of *Vitis amurensis*. Phytochemistry 2000; in press.
9 Yue TL, Gu JL, Lysko PG, Sponer G, Cheng HY, Ruffolo RRJ, *et al*. Antioxidant activity of a β-adrenoceptor antagonist, carazolol: comparison with propranolol and carvedilol. Pharmacol Commun 1995; 7: 11 – 9.

山葡萄籽多酚类化合物体外抗氧化活性的研究

王建农[1]，陈英杰[2]，羽野芳生[3]，野村太郎[3]，谭仁祥 (南京大学生命科学学院医药生物技术国家重点实验室，南京 210093，中国；[2]沈阳药科大学中药系，沈阳 110015，中国；[3]*Faculty of Pharmaceutical Science, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan*)

关键词 山葡萄；葡萄科；抗氧化剂；儿茶素；原花青素 B_2；原花青素 B_5；原花青素 B_5-3′-O-gallate；山葡萄素

目的：本文对分自山葡萄籽中的五种多酚类化合物(+)-儿茶素、原花青素 B_2、原花青素 B_5、原花青素 B_5-3′-O-gallate 和山葡萄素进行抗脂质过氧化研究. **方法**：采用体外抗小鼠肝匀浆脂质过氧化的方法对这些化合物的抗氧化活性进行评价. **结果**：(+)-儿茶素，原花青素 B_2，原花青素 B_5，原花青素 B_5-3′-O-gallate 和山葡萄素等五种化合物对小鼠肝匀浆过氧化脂质生成的半数抑制浓度(IC_{50})分别为 0.47、0.25、0.10、0.02 及 0.03 mmol/L，阳性对照维生素 E 的 IC_{50}为 0.13 mmol/L. 对这些化合物的构效关系进行的初步分析表明：带有没食子酰基的多酚具有更强的抗氧化活性，二聚体的抗氧化活性均比单体(+)-儿茶素的活性强. 另外，4→6 连接的二聚体比 4→8 连接的二聚体具有更强的抗氧化活性. **结论**：原花青素 B_5、原花青素 B_5-3′-O-gallate 及山葡萄素的抗氧化活性都比维生素 E 强，且这类多酚的抗氧化活性强弱与取代类型及聚合方式密切相关.

(责任编辑 吕 静)