Arch. Pharm. Res. Vol. 17, No. 1, pp. 42-44, 1994

Note

Antitumor Activity of some Phenolic Components in Plants

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(Received October 21, 1993)

The activity-guided fractionation of some medicinal plants led to yield five kinds of natural stilbene compounds namely 3,5-dihydroxy-4'-methoxystilbene(**I**), rhapontigenin(**II**), resveratrol (**III**), rhaponticin(**IV**) and piceid(**V**) and two common flavonoids, apigenin(**VI**) and luteolin(**VII**) as active principles of the antitumor property, in vitro, against five kinds of human tumor cell lines, A-549, SK-OV-3, SK-MEL-2, XF-498 and HCT15.

Key words: Resveratrol, Rhapontigenin, Phaponticin, Piceid, Apigenin, Luteolin, Antitumor

INTRODUCTION

We have investigated for the isolation of antitumeric active components from natural resources especially from medicinal plants. On this purpose, more than one hundred kinds of medicinal plants were extracted and partitioned serially with $CHCl_3$, EtOAc and H_2O, respectively, and each fractions were examined for the antitumoral activity *in vitro*, on the basis of the direct cytotoxicity of them against five kinds of cultured human tumor cell lines, *i.e.*, A-549 (non small cell lung), SK-OV-3 (ovarian), SK-MEL-2 (skin), XF498 (CNS) and HCT15 (colon). On this survey, the EtOAc fractions of the rhizomes of *Rheum undulatum* (Polygonaceae) and *Polygonum cuspidatum* (Polygonaceace) and that of the flower of *Chrysanthemum indicum* (Compositae) were exhibited an marked antitumor activity against examined human tumor cells. The present paper reports the antitumor activity of active principles isolated from the plants against cultured human tumor cell lines *in vitro*.

MATERIALS AND METHODS

^{1}H-NMR spectra were run at 300 MHz recorded by Bruker-AM-300. EIMS (70 eV) were taken with a direct inlet recorded by GC-MS QP-100 (Shimadzu) spectrometer. The reference compound for the antitumor activity, quercetin(**VIII**) and rutin(**IX**) were purchased from *Aldrich*. Tumor cells for the experiments were obtained from the National Cancer Institute (NCI), USA, which were currently used in the NCI's *in vitro* anticancer-drug screening. Stock cultures were grown in T-25 (Falcon) flasks containing 10 ml of RPMI-1640 medium with glutamine, sodium bicarbonate and 5% fetal calf serum. Cells were dissociated with 0.25% trypsin and 30 mM 1,2-cyclohexanediaminetetraacetic acid in PBS just before transferring for experiment.

Antitumor Test *in vitro*

All experimental procedures were followed up according to the NCI's protocol (Skehan *et al.*, 1990). Detailed were described on the previous paper (Ryu *et al.*, 1992).

Extraction and Isolation

All commercially available plant materials for the experiment were purchased at market and were refluxed with MeOH for 3 hrs. The MeOH extract of each plant material was partitioned with $CHCl_3$, EtOAc, BuOH and H_2O, successively. Then, the activity of resultant fractions were assessed *in vitro*, respectively. One of active ones, the EtOAc fraction of the rhizome of *Rheum undulatum* was subjected to divide into 4 portions (fr.A-fr.D) by the SiO_2 gel column chromatography eluted with CH_2Cl_2/MeOH, of which the fr.D was the most active portion. The fr. D was repeated with silica gel chromatography according to the guidance of the activity and finally led to yield three active constituents **I**, **II** and **IV**. By the same manner, the EtOAc fraction of the rhizome of *Polygonum cuspidatum* resulted in

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two active principles **III** and **V**, and that of the flower of *Chrysanthemum indicum*, **VI** and **VII**.

Compound **I** (3,5-Dihydroxy-4'-methoxystilbene). yield 0.075%, white needle (MeOH/H_2O), mp. 175-178 ℃, UV: λ_{max} (MeOH); 320, 307, MS: m/z (rel. int.); 242 (M^+, 100), 241(15), 115(14), ^{1}H-NMR (DMSO-d_6, δ): 7.51(2H, d, J=8.8 Hz, 2',6'-H), 6.93 (2H, d, J=8.8 Hz, 3',5'-H), 7.02, 6.82 (each 1H, d, J=16 Hz, olefinic-H), 6.40 (2H, d, J=2.0 Hz, 2,6-H), 6.13 (1H, m, 4-H), 3.77 (3H, s, -OCH_3).

Compound **II** (3,3',5-Trihydroxy-4'-methoxystilbene, rhapontigenin). yield 0.55%, white needle (MeOH/H_2O), mp. 195-198℃, UV: λ_{max} (MeOH); 325, 302, MS: m/z (rel. int.); 258 (M^+, 100), 225(17), 197(64), 129(14), 115 (25), ^{1}H-NMR (DMSO-d_6, δ): 7.00 (1H, d, J=1.6 Hz, 2'-H), 6.96 (1H, dd, J=8.8, 1.6 Hz, 6'-H), 6.92 (1H, d, J=8.0 Hz, 5'-H), 6.91 and 6.62 (each 1H, d, J=16 Hz, olefinic-H), 6.38 (2H, d, J=2.0 Hz, 2,6-H), 6.12 (1H, m, 4-H), 3.78 (3H, s, -OCH_3).

Compound **III** (3,4',5-Trihydroxystilbene, resveratrol). yield 0.45%, white needle (MeOH/H_2O), mp. 255-260 ℃, UV: λ_{max} (MeOH); 310, 300, MS: m/z (rel. int.); 228 (M^+, 100), 227(25), 211(16), 182(22), 115(15), ^{1}H-NMR (CD_3OD, δ): 7.35 (2H, d, J=8.8 Hz, 2',6'-H), 6.79 (2H, d, J=8.8 Hz, 3',5'-H), 6.98 and 6.74 (each 1H, d, J=16 Hz, olefinic-H), 6.46 (2H, d, J=2.0 Hz, 2,6-H), 6.17 (1H, m, 4-H).

Compound **IV** (3,3',5-Trihydroxy-4'-methoxystilbene-3-O-β-D-glucoside, rhaponticin, ponticin). yield >1.0%, pale yellowish needle in MeOH, mp. 245-249℃, UV: λ_{max} (MeOH); 325, 302, MS: m/z (rel. int.); 420 (M^+, 1), 258(35), 197(26), 181(4), 129(11), 115(16), ^{1}H-NMR (DMSO-d_6, δ): 7.02 (1H, d, J=1.6 Hz, 2'-H), 6.97 (1H, dd, J=8.8, 1.6 Hz, 6'-H), 6.72 (1H, d, J=8.0 Hz, 5'-H), 7.04 and 6.84 (each 1H, d, J=16 Hz, olefinic-H), 6.72 and 6.57 (each 1H, brs, 2,6-H), 6.34 (1H, m, 4-H), 4.30 (1H, d, J=6.8 Hz, anomeric-H), 3.78 (3H, s, -OCH_3), 3.2-3.9 (5H, m, glucose-H).

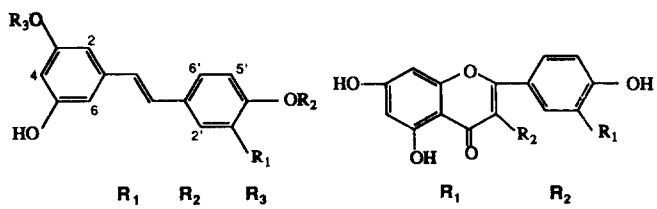
Compound **V** (3,4',5-Trihydroxystilbene-3-O-β-D-glucoside, piceid). yield >1.0%, colorless needle in MeOH. mp. 225-228℃, UV: λ_{max} (MeOH); 310, 300., MS: m/z (rel. int.); 390 (M^+, 2), 228(100), 211(11), 181 (20), 115(15), ^{1}H-NMR (DMSO-d_6, δ): 7.40 (2H, d, J=8.8 Hz, 2',6'-H), 6.75 (2H, d, J=8.8 Hz, 3',5'-H), 7.07 and 6.82 (each 1H, d, J=16 Hz, olefinic-H), 6.70 (1H, d, J=2.0 Hz, 6-H), 6.57 (1H, d, J=2.0 Hz, 2-H), 6.34 (1H, m, 4-H), 4.80 (1H, d, J=7.5 Hz, anomeric-H), 3.2-4.0 (5H, m, glucose-H).

Compound **VI** was identified as the apigenin (4',5,7-trihydroxyflavone) and **VII** as luteolin (3',4',5,7-tetrahydroxyflavone), respectively by the direct comparison of chemical properties with those of authentic samples.

RESULTS AND DISCUSSION

The activity-oriented fractionation of the MeOH extract of the rhizome of *Rheum undulautum* was led us to the isolation of three natural polyhydroxystilbene components **I**, **II** and **IV** as the active principles for the antitumor property of the plant material. By the same way, the MeOH extract of the rhizome of *Polygonum cuspidatum* also yielded two polyhydroxystilbenes **III** and **V** (Fig. 1). The antitumor activity of them against five kinds of cultured human tumor cell lines *in vitro* were summarized on Table I. Hydroxystilbenes **I**, **II** and **IV** were exhibited an excellent activity with a similar potency regardless of the structural difference between each other on the substitution pattern of hydroxyl groups, whereas the corresponding glucosides **III** and **V** were shown to have a poor activity, probably due to their poor solubility in water. Therefore, it seemed that the trans-stilbene skeleton of them rather than the substituent groups was more responsible to the antitumor activity. It has been reported that naturally occurring hydroxystilbenes exhibited many diverse biological activities such as phytogrowth-inhibitory activity (Inamori *et al.*, 1984), antimicrobial activity (Inamori *et al.*, 1985), the inhibitory effect on MAO (monoamine oxidase) and AADC(aromatic l-aminoacid decarboxylase) of rat brain (Ryu *et al.*, 1988; Han *et al.*, 1990), antithrombotic activity (Kimura *et al.*, 1985) including the inhibitory action on the platelet aggregation (Goda *et al.*, 1987) and the vasodilator and hypotensive activity (Kubo *et al.*, 1984). Concerned to the antitumor activity of them, Pettit *et al.* (1987) reported that combretastatin A-1 and its congeners, kinds of natural *cis*-stilbene were shown to possess an inhibitory activity on microtubule assembly *in vitro*. Recently, Mannila *et al.* (1992) reported that some stilbenes from Picea abies were exhibited a mild cytotoxic activity against murine leukaemia L1210. Another point of interest, it was wellknown that several synthetic stilbenes such as diethylstilbestrol, tamoxifen and toremifen had been used widely for the treatment of some cancers.

Other two active components from *Chrysanthemum*



	R_1	R_2	R_3
I	-H	-Me	-H
II	-OH	-Me	-H
III	-H	-H	-H
IV	-OH	-Me	-glu
V	-H	-H	-glu

	R_1	R_2
VI	-H	-H
VII	-OH	-H
VIII	-OH	-OH
IX	-OH	-O-rutinose

Fig. 1. Antitumeric stilbenes and flavonoids from plants.

Table I. *In vitro* antitumor activity of phenolic components isolated from medicinal plants expressed as ED_{50}*

compound	tumor cell line				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
I	3.8	5.7	3.2	5.6	6.7
II	3.9	4.6	3.1	7.9	3.0
III	3.5	3.7	2.4	3.8	3.5
IV	48 5	>50	35.4	>50	>50
V	50.4	>50	42.8	>50	>50
VI	7.8	7.5	4.3	6.0	6.5
VII	3.4	4.4	1.9	2.8	4.4
VIII	5.8	6.3	4.7	6.0	4.8
IX	>50	>50	>50	>50	>50

*ED_{50} value of the compound against each tumor cell line, which was defined as a concentration (μg/ml) that caused 50% inhibition of the cell growth *in vitro*

indicum was identified as common flavones, apigenin **VI** and luteolin **VII** (Fig. 1). Even though each of them was a typical compound of naturally occurring flavonoids and also most widely abundant in plant kingdom, they were exhibited an activity against each tumor cells *in vitro* by our bioassay system. Besides, the reference compound, quercetin, which was also a kind of typical flavone, exhibited an activity with a similar potency as those of **VI** or **VII**, whereas, rutin, a glycoside of quercetin, showed a negligibly poor activity (Table I). Therefore, It was still questionable whether the activities of **VI** and **VII** including that of the quercetin was a reliable or just a simple false positive result due to our bioassay system, because these compounds were generally known as a kind of carcinogen rather than as antitumor agents. However, a lot of reports had been presented that some kinds of flavonoids including **VI** and **VII** were found to show the cytotoxicity against various cell lines *in vitro* and *in vivo* (Suolinna *et al.*, 1974; Ahn *et al.*, 1985; Lee *et al.*, 1981). Especially, Lee *et al.* (1981) had reported that tricin (5,7-dihydroxy-3',4',5'-trimethoxyflavone) and kaempferol-3-O-β-D-glucopyranoside were found to exhibit a significant inhibitory activity *in vivo* against P-388 lymphocytic leukemia growth in mice (T/C=174 and 130).

ACNOWLEDGEMENT

This research was supported by the special research project on the development of new pharmaceuticals from natural products, Ministry of Science and Technology, Korea.

REFERENCES CITED

Ahn, B. Z., Ryu, S. H. and Pack, M. Y., The cytotoxic principle of Scutellariae Radix against L1210 cell. *Planta Medica,* 462 (1985).

Goda, Y., Shibuya, M. and Sankawa, U., Inhibitors of the arachidonate cascade from *Allium chinense* and their effect on *in vitro* platelet aggregation. *Chem. Pharm. Bull.,* 35, 2668-2674 (1987).

Han, Y. N., Ryu, S. Y. and Han, B. H., Antioxidant activity of resveratrol closely correlates with its Monoamine oxidase-A inhibitory activity. *Arch. Pharm. Res.,* 13, 132-135 (1990).

Inamori, Y., Kubo, M., Kato, Y., Yasuda, M., Baba, K. and Kozawa, M., The antifungal activity of stilbene derivative. *Chem. Pharm. Bull.,* 32, 801-804 (1984).

Inamori, Y., Kubo, M., Kato, Y., Yasuda, M., Tsujibo, H., Baba, K. and Kozawa, M., The biological activities of 3,4-O-isopropylidene-3,3',4,5-tetrahydroxystilbene. *Chem. Pharm. Bull.,* 33, 2904-2909 (1985).

Kimura, Y., Okuda, H. and Arichi, S., Effects of stilbene derivatives on arachidonate metabolism in leukocytes. *Biochim. Biophys. Acta.,* 837, 209-212 (1985).

Kubo, M., Inamori, Y., Kato, Y., Yasuda, M., Baba, K. and Kozawa, M., The coronary vasodilator action of stilbene derivatives on guinea-pig heart and a hypotensive effects on rat. *Yakugaku Zasshi,* 104, 819-823 (1984).

Lee, K. S., Tagahara, K., Suzuki, H., Wu, R. Y., Haruna, M., Hall, I. H. and Huang, H. C., Antitumor agents. 49. Tricin, Kaempferol-3-O-β-D-glucopyranoside and (+)-nortrachelogenin, antileukemic principles from Wikstroemia indica. *J. Nat. Prod.,* 44, 530-535 (1981).

Mannila, E. and Talvite, A., Stilbenes from Picea abies Bark. *Phytochemistry,* 31, 3288-3289 (1992).

Pettit, G. R., Singh, S. B., Niven, M. L., Hamel, E. and Schmidt, J. M., Isolation, structure, and synthesis of combretastatins A-1 and B-1, potent new inhibitors of microtubule assembly, derived from *Combretum caffrum. J. Nat. Prod.,* 50, 119-131 (1987).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer. Inst.,* 82, 1107-1112 (1990).

Suolinna, E. M., Lang, D. R. and Racker, E., Quercetin, an artificial regulator of the high aerobic glycolysis of tumor cells. *J. Natl. Cancer Inst.,* 53, 1515-1519 (1974).

Ryu, S. Y., Han, Y. N. and Han, B. H., Monoamine Oxidase-A inhibitors from medicinal plants. *Arch. Pharm. Res.,* 11, 230-239 (1988).

Ryu, S. Y., Choi, S. U., Lee, C. O. and Zee, O. P., Antitumor activity of Psoralea corylifolia. *Arch. Pharm. Res.,* 15(4), 356-359 (1992).