Natural Product Sciences
12(4) : 175-192 (2006)

Biological Characterization of the Chemical Structures of Naturally Occurring Substances with Cytotoxicity

Hee-Juhn Park[1], Hyun-Ju Jung[1], Kyung-Tae Lee[2], and Jongwon Choi[3]

[1]Division of Applied Plant Sciences, Sangji University, Wonju 220-702, Korea
[2]College of Pharmacy, Kyung-Hee University, Seoul 130-701, Korea
[3]College of Pharmacy, Kyungsung University, Pusan 608-736, Korea

Abstract – Screening for the cytotoxicity from plant origin is the first stage for anti-cancer drug development. A variety of terpenoids with exomethylene, epoxide, allyl, α,β-unsaturated carbonyl, acetylenes, and α-methylene-γ-lactone induces apoptosis and/or differentiation as well as cytotoxicity through the ROS signal transduction pathways. These are found among monoterpenes, sesquiterpenes, triterpenes, flavonoids, coumarins, diarylheptanoids, and even organosulfuric compounds. The most essential characteristics of natural cytotoxic substances is to possess the strong electrophilicity that is susceptible to nucleophilic biomolecules in the cell. Thiol-reductants and superoxide dismutase can block or delay apoptosis. Thus, ROS and the resulting cellular redox-potential changes can be parts of the signal transduction pathway during apoptosis. Disturbance of the balance of oxi-reduction by the pigment of natural quinones also caused the induction of the differentiation and apoptosis. Saponins with the cytotoxicity are restricted to their monodesmosides, rather than to bisdesmosides. Those saponins exhibited calcium ion-mediated apoptosis in addition to cytotoxicity whereas they showed also differentiation without extracellular calcium ion. The properties on cytotoxicity, apoptosis, and differentiation were assumed to depend on resultant oxidative stress to the cells. In this review, we describe a spectrum of cytotoxic compounds with various action mechanisms.
Keywords – cytotoxicity, natural products, apoptosis, differentiation, reactive oxygen species (ROS), oxidative stress

Introduction

Screening of naturally occurring substances and synthetic chemicals for their cytotoxicity can be a fundamental step for the development of anticancer agent. Recognition of obvious structure-cytotoxicity relationship on naturally occurring substances will allow to avoid unnecessary laboratory works. Oriental herb drugs often contain chemopreventive ingredients capable of being involved in anti-lipid peroxidation, anti-mutagenicity and anti-aging. Cells resist bad circumstances through genetic expressions, but more severe state lead to cell's apoptosis (programmed cell death) and mutagenicity (Yusuf, 1997). Reactive oxygen moiety (ROS), electrophilic substances and activated xenobiotics could make those circumstances and resultantly be toxic to the cell. These can combine with bases of DNA or result in radical chain reaction in the living system, and therefore they are fatal to the cell (Singer, 1975). It is of interest that there are structural common aspects between the natural products inducing mutagenicity, apoptosis and the inhibition of inducible nitric oxide. In this article, we discuss mainly on the common aspects of the electrophilic cytotoxic natural compounds with cytotoxicity.

*Author for correspondence
Fax: +82-33-730-0564; E-mail: hjpark@sangji.ac.kr

Structure-Activity Relationship

Structure of cytotoxic terpenoids – Terpenoids are a group of the most unique structure in plant kingdom. Mevalonate started from acetyl Co-A by biogenetic pathway can produce a tremendous variety of terpenoids using head-to-tail reaction between isopentenyl pyrophosphate and dimethylallyl pyrophosphate. Terpenoid that agree to the isoprene rule includes a number of cytotoxic compounds. Though other compounds have anti-fungal, anti-viral, or anthelmintic, respectively (Harborne *et al.*, 1993), the most characteristic and the most comprehensively studied region of the bioassay is cytotoxicity. We have isolated costunolide (**1**) of sesquiterpene lactone in a

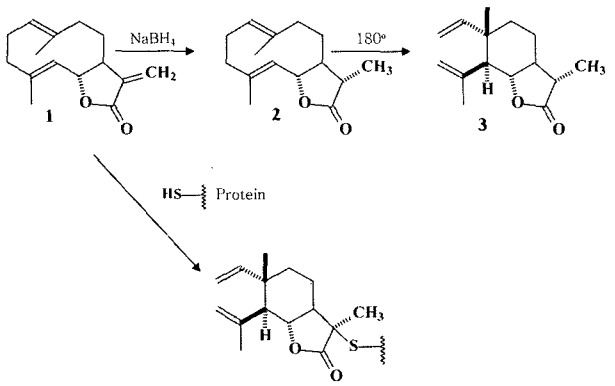


Fig. 1. Proposed chemical change by Michael addition of protein SH to **1** (costunolide) and chemical transformation of costunolide to **2** (dihydrocostunolide) and **3** (saussurea lactone).

high yield from *Magnolia sieboldii.* (Park *et al.*, 2001) $NABH_4$ reduction of cytotoxic costunolide with cytotoxicity produced dihydrocostunolide (**2**) with inactive very low cytotoxicity (Choi *et al.*, 2001). (Fig. 1) This explains exomethylene of costunolide is its essential moiety for the cytotoxicity. On treatment of heat to dihydrocostunolide, its structure was changed to that of saussurea lactone (**3**). Although this structure has a 1,2-trans-divinyl moiety, the compound did not show any cytotoxicity. The compounds that have no binding capability to any of cellular nucleophilic antioxidant generally do not show cytotoxicity, in spite of the existence of divinyl moiety in the molecule. Since the compounds have no binding capability to any of cellular nucleophilic antioxidative proteins, they did not show cytotoxicity, in spite of the existence of divinyl moiety in the molecule. On the other hand, costunolide was known to have chemically unstable structure. Many readily reactive compounds possess unstable partial structure and proceeds in the exergonic reaction. From no cytotoxicity of chemically stable saussurea lactone produced from thermal lysis, we can presume many aspects on the structure-activity relationship. Most sesquiterpene lactone glycosides do not show cytotoxicity in any of reports, in spite of the existence of α-methylene-γ-lactone, which suggest that sugar moieties may prevent the affinity of cellular reaction. One of predictable preventions could be hard traverse across cell membrane because of its considerably higher polarity. Most highly cytotoxic terpenoids can be found in the relatively hydrophobic ones. Lower level of cytotoxic compounds in the cell reduces the possibility of molecular collision for reaction. Although those glycosides do not exhibit cytotoxicity, they can produce the active sesquiterpene lactone by the removal of glycoside moiety. Therefore, the most significant characteristics of the cytotoxic sesquiterpenoids in structure

Table 1. Cytotoxic activity of costunolide (**1**) on cancer cell growth *in vitro*

cell line	origin	IC_{50} (μM)[a]	
		costunolide	cisplatin
Colon 26	colon adenocarcinoma	17.2 ± 1.4	12.3 ± 0.9
3LL Lewis	lung carcinoma	19.3 ± 1.9	15.5 ± 1.1
J82	bladder carcinoma	16.4 ± 2.5	16.1 ± 2.9
T24	bladder carcinoma	21.9 ± 3.5	21.9 ± 3.0
HL-60	leukemia	9.5 ± 0.8	9.5 ± 1.6

[a] IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number.
[b] The values represent the mean ± S.D of three independent experiments.
* $P < 0.05$, ** $P < 0.01$

should be attributed to the partial structure of high affinity and to hydrophobic entire structure. However, we can meet the cytotoxic terpenoids with epoxide as another functional group, which remains no doubt for suffering from very sensitive reaction with sulfhydryl biomolecules. On consideration of cytotoxicity, apoptosis and differentiation of magnolialide in our previous report, it can be observed that this eudesmanolide with a hydroxyl group shows a little higher polarity and a little less potent cytotoxicity than costunolide (Choi *et al.*, 2001; Park *et al.*, 2000). The less activity of magnolialide (**4**) can be attributed to the less rapid traverse of magnolialide across cell membrane than costunolide. Simple diffusion across cell membrane allows easier passage of hydrophobic substances. This fact was observed in our continual study of the cytotoxicity. On the comparisions of cytotoxicity of intermedeol (**5**), 6-oxoeremophilenolide (**6**) and ether extract obtained from *Ligularia fischeri* var. *spiciformis* methanolic extract, a relatively lower cytotoxicity of 6-oxoeremophilenolide, relatively higher cytotoxicity of intermedeol and medium cytotoxicity of the ether extract was found (Park *et al.*, 2000). (Fig. 2, Table 2) Cytotoxic activities of intermedeol bearing an exomethylene were shown to be much more potent than those of 6-oxoeremophilenolide having α-methyl-α,β-unsaturated-γ-lactone. Intermedeol contains simply an exomethylene, not a α-methylene-γ-lactone. Therefore, it is obvious that a lactone ring system serve the potency of exomethylene in sesquiterpene lactone with cytotoxicity. Additional terpenoids, spiciformisin a (**7**), spiciformisin b (**8**) and monocyclosqualene were isolated from the ether fraction of *L. fischerii* var. *spiciformis* for further research to obtain other cytotoxic compounds (Lee *et al.*, 2001). Of spiciformisins, both acyclic diterpenes with each of conjugated dienyl groups, spiciformisin a showed significant cytotoxicities against cancer cells. However, the

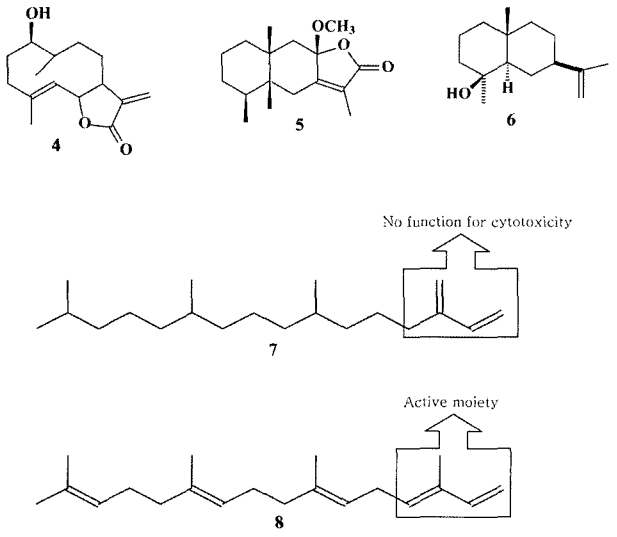


Fig. 2. Structure of terpenoids {**4** (magmolialide), **5** (6-oxoeremophilenolide), **6** (intermedeol), **7**} and active moiety of spiciformisins a (**7**) and b (**8**).

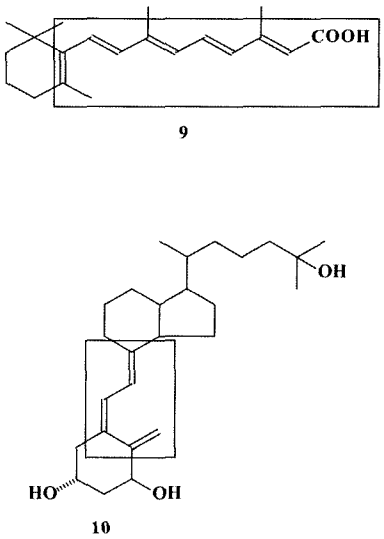


Fig. 3. Active moiety of both differentiation inducers, 1,25-dihydroxyvitamin D_3 (**9**) and retinoic acid (**10**).

Table 2. Cytotoxic activities of **4**, **5** and diethylether fraction from *Ligularia* species on tumor cell growth

cell line	IC_{50} (μg/ml)[a]			
	diethylether fr.	**5**	**4**	cisplatin
HL-60	28.8	242.5	25.7	5.3
U-937	38.4	53.6	15.9	6.6
P-388	66.5	184.0	13.6	6.9
L-1210	44.4	298.2	12.4	1.0
HepG-2	50.0	> 400	77.0	5.2
Vero	70.2	374.5	63.4	4.4
SNU-C5	41.6	381.3	74.1	8.3
T-24	126.5	> 400	77.0	4.1
J-82	143.0	355.0	77.3	5.6

[a]IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number.
The values represent the mean of three independent experiments.

other diterpene showed no cytotoxicity, which explains no affinity to nucleophilic antioxidant in the cell. The trans-conjugation of double bonds may responsible for the cytotoxicity, in addition to the partial symmetric structure of diexomethylene system. The preferable structure of *cis*-conjugated system in 1,3-butadiene has been known (Park *et al.*, 2001). When we examine the partial structure of 1,3-butadiene in spiciformisin a, this structure which can be situated in a resonance state shows a partial symmetricity. This chemically characteristic structure might make spiciformisin a lose affinity capability to sulfhydryl biomolecules.

Our assumption agreed to the structural inspection on 1,25-dihydroxyergocalciferol (**9**) and retinoic acid (**10**), both differntiation inducers. (Fig. 3) In spite of no report on glutathione-affinity of them, we believe that they could combine with sulfuhydryl biomolecule from the existence of conjugated polyenyl carbonyl or conjugated trienyl exomethylene in the molecules. By these structures, they may need low activation energies for the covalent bonding. Costunolide actually exhibited very similar effects with the known differentiation inducer in both apoptosis- and differentiation-inducing ability. Again, a very low concentration of costunolide significantly decreased nitric oxide induced by lipopolysaccharide (LPS) in macrophage 7734.1 (Park *et al.*, 1996). The concentrations higher than IC_{50} value for inducible nitric oxide inhibition caused differentiation of leukemic cells while the concentrations higher than IC_{50} value for the differentiation induced apoptosis (Kim *et al.*, 1997). Throughout many reports on cytotoxicity, differentiation and apoptosis of natural products, the IC_{50} values for the activities could be explained like above. Conclusively, a part of signal tranduction, in particular earlier stage of them, might be shared between those three activities. Our research on signal transduction pathway of costunolide revealed that DNA laddering induced by costunolide is caused by the inhibition of anti-apoptotic protein *bcl*-2, mitochondrial cytochrome c release and further caspase-3 activation (Park *et al.*, 2001). All the signal transduction by costunolide was nearly blocked by excess concentration of glutathione. Therefore, it was indicated that binding of intracellular antioxidant glutathione to the sesquiterpene

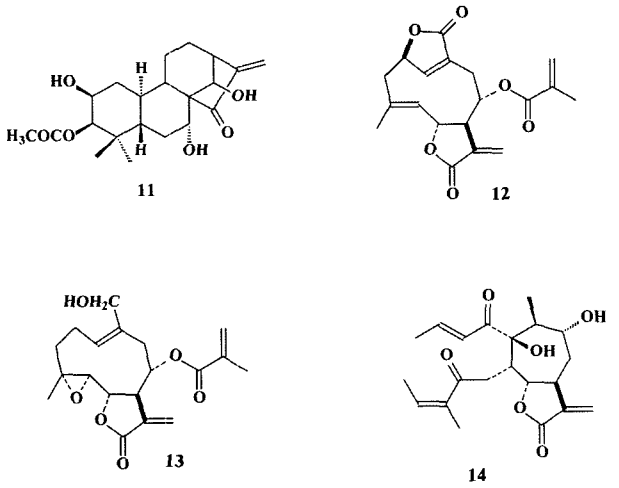


Fig. 4. Structure of terpenoids **11-14**. {**11** (leukamenin A), **12** (deoxyphantopin), **13** (tomenphantin A), **14** (9α-hydroxy-seco-ratiferolide-5α-O-angelate)}.

lactone could be the earliest stage for the signal transduction. On the other hand, treatment of cyclosporin A capable of scavenging reactive oxygen intermediates restored the cell cycle to the normal group (Lee *et al.*, 2001). These suggested that the decrease of intracellular glutathione might cause the signal transduction together with the involvement of reactive oxygen intermediates in the signal transduction. It was believed that most of cytotoxic terpenoids with the active partial structure might follow the signal tranduction discussed above.

From a large volume of reports on the cytotoxicity of terpenoids, our viewpoint could be supported by those active moieties. Many cytotoxic diterpenoids and their structures were established together with the structure and the IC_{50}s of the most cytotoxic leukamenin A (**11**) with ent-kaurene skeleton (Fujita *et al.*, 1988). (Fig. 4) Although α-methylene-γ-lactone is easily found in sesquiterpene lacotones, leukemanin A have the same moiety for cytotoxicity as in costunolide. We assume that a hydroxyl next to the ring enhances the susceptibility for the binding with glutathione and further contribute to the potent cytotoxicity. A germacranolide (**12**) isolated by Zhang (Zhang *et al.*, 1986) has characteristically not only the two exomethylenes but also the two lactone rings. Another germacranolide (**13**) isolated by Hayashi (Hayashi *et al.*, 1999) has the two exomethylenes together with the epoxide capable of showing electrophilicity. One of xantholide (**14**), 9α-hydroxy-seco-ratiferolide-5α-O-angelate, isolated by Cui (Cui *et al.*, 1999) has a α-methylene-γ-lactone and opened in A-ring.

Cytotoxic phenylpropanoids – Anti-fungal assay performed by Bang *et al.* (Bang, 2000) demonstrated that

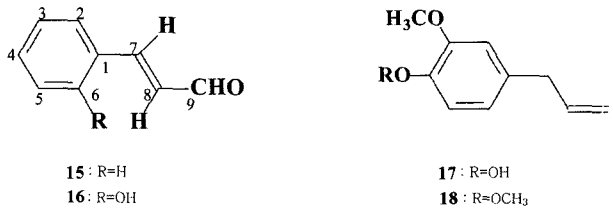


Fig. 5. Structure of **15** (cinnamaldehyde), **16** (2-hydroxycinnamaldehyde), **17** (eugenol) and **18** (methyleugenol).

Cinnamomum cassia was the most potent anti-fungal herbal drug among hundreds of plants collected. The active compounds cinnamaldehyde (**15**) was isolated from that plant and the mechanism was attributed to decrease of cell wall biosynthesis by chitin synthase inhibition (Bang and Lee, *et al.*, 2000). Actually, naturally occurring anti-fungal substances have mostly the cytotoxicity to human cancer cells because they are all eucaryotic cells. Thus the binding ability to glutathione (van Lerzel *et al.*, 1997), mutagenicity (Azizan *et al.*, 1995), anti-mutagenicity (Fiorio *et al.*, 1994) are often correlated to anti-fungal and cytotoxicity (Lee *et al.*, 1999). 2-Hydroxycinnamaldehyde (**16**) isolated from *Cinnamomum cassia* was reported to have *in vivo* anti-tumor activity through the inhibition of *ras* protein (Lee *et al.*, 1999). We continued to study on the extraction of the essential oil of *C. cassia*, (Fig. 5) cytotoxicity and signal transduction to apoptosis because of the probability to combine with intracellular glutathione. Our successive studies revealed the glutathione depletion in the cell and certainly induction of apoptosis (Ka *et al.*, 2001). Strong nucleophilic antioxidant N-acetyl L-cysteine blocked the apoptosis indicating the covalent bonding by Michael addition. Organic chemical assumption suggested that alkylthio group should be bound with C-7 of cinnamaldehyde and proton to C-9. Based on the negative Michael addition of aldehyde itself to glutathione, it could be suggested that polyenyl moiety of cinnamaldehyde serves the binding capability. The unique structure of cinnamaldehyde might decrease the activation energy for the reaction. No cytotoxicity of cinnamoyl chloride reminded us that aldehydic proton in cinnamaldeyde play a more important role in that binding than acid chloride in the former compound (data not shown). In addition, 2-hydroxycinnamaldehyde exhibited more potent cytotoxicities than cinnamaldehyde. Taken together, it was found that the substitution of OH at C-2 enhances the Michael addition.

The most important condition for the clinical application of cinnamaldehyde is not to cause lipid peroxidation, based on its electrophilicity. In some reports, cinnamaldehyde was shown to have mutagenicity. Therefore, we investigated

Table 3. Effect of cinnamaldehyde (**15**) isolated from *C. cassia* essential oil (CC-oil) on hepatic drug-metabolizing enzymes in rats

group dose	normal	cinnamaldehyde (15, mg/kg)	
	0	25	50
MDA[2)]	$25.3 \pm 0.51^{1)a}$	20.4 ± 0.29^{b}	18.6 ± 0.18^{c}
glutathione S-transferase[3)]	206.8 ± 9.43^{a}	119.4 ± 11.01^{a}	118.7 ± 8.96^{a}
glutathione[4)]	2.19 ± 0.18^{a}	2.31 ± 0.20^{a}	2.27 ± 0.24^{a}
epoxide hydrolase[5)]	12.3 ± 0.31^{a}	23.6 ± 0.24^{b}	28.2 ± 0.19^{c}
aminopyrine demethylase[6)]	2.86 ± 0.19^{a}	1.73 ± 0.13^{b}	1.06 ± 0.15^{c}
aniline hydroxylase[7)]	0.58 ± 0.06^{a}	0.36 ± 0.07^{b}	0.29 ± 0.09^{b}
aldehyde oxidase[8)]	1.38 ± 0.11^{a}	1.44 ± 0.10^{a}	1.38 ± 0.17^{a}
xanthine oxidase[9)]	2.34 ± 0.90^{a}	2.47 ± 0.12^{a}	2.50 ± 0.17^{a}

Rats were intraperitoneally injected daily for seven days and animals were decapitated 24 h after the last injection. [1)]Vlaues are mean ± S.D. for six experiments. Values with same superscript letter are not significantly different in each row ($p < 0.05$); Unit: [2)]nmol/g of tissue [3)]1,2-dinitro-4-nitrobenzene nmol/mg protein/min [4)]μmol/g of tissue [5)]nmol/g of tissue [6)]HCHO nmol/mg protein/min [7)]p-aminophenol nmol/mg protein/min [8)]2-pyridone nmol/mg protein/min [9)]uric acid nmol/mg protein/min.

the lipid peroxide and hepatic drug-matabolizing enzymes 7 days after the treatment of cinnamaldehyde and the essentaial oil of *C. cassia* to rats. These substances inhibited the formation of lipid peroxide and the activity of glutathione S-transferase by less than those of normal rats (Choi and Lee *et al.*, 2001). In addition, cytochrome P_{450} enzyme activity was considerably decreased, which enzyme could generate reactive oxygen species. (Table 3) Other enzymes involving in antioxidation were changed to a preferable direction for anti-lipid peroxidation. We could find here that cinnamaldehyde conjugates with glutathione either in unicellular organism or in higher organism. Furthermore, higher organism could increase hydrophilicity through the binding and excrete to urine. Therefore, cinnamaldehyde could be valuable for cancer chemopreventives *in vivo* rather than mutation-inducing agent. A typical phenylpropanoid caffeic acid was reported to have not only antioxidant action on LDL oxidation (Andreasen *et al.*, 2001) but also apoptogenic activity (Chen *et al.*, 2001). Caffeic acid has both a partial structure responsible for antioxidant action, *o*-dihydroxyphenyl, and prooxidative one for α,β-unsaturated carbonyl. In spite of no interpretation on their biological activities, the structure may represent those effects. Many of phenylpropanoids actually show antioxidative effect including free-radical scavenging effect. Therefore, it is believed that most biological activities such as antibacterial, anti-tumor, antiviral or anti-fungal are dependent on the properties either of their electrophilicity or of antioxidation.

Very well known eugenol (**17**) is used in dental department as sterilizing purposes and it has also analgesic and local anesthetic effects (Han, 1992). Meanwhile, it has a very weak cytotoxicity rather than no activity but considerably potent free-radical scavenging effect. Interestingly, methylation (**18**) of eugenol decreased free-radical scavenging effect but failed to show a considerable change of cytotoxicity (Park and Park *et al.*, 2001). Therefore, it could be suggested that phenolic OH of eugenol is closely associated with free-radical scavenging effect, though exomethylene would be responsible for the cytotoxicity. Intraperitoneal treatment of eugenol to the normal rat showed a different pattern of hepatic lipid peroxide content and hepatic drug-metabolizing activity than those of cinnamaldehyde. (Table 4) The trend of an example of eugenol treatment is similar to those of xenobiotics. Although various bioactivities of eugenol has been known, it could be commented that there is difficulty in the development of a cancer chemopreventive including all other systemic uses for the chemotherapy. Based on the above discussions, we have to recognize the importance of these aspects to the development of cancer chemopreventives of phenylpropanoids.

Volatile organosulfuric compounds – It is widely known that many Allium species plants are known to have organosulfuric compouns. In particular, garlic, bulbs of *Allium sativum* have these components in addition to its famous anti-tumour effect (Matsuura). The components of a steroidal saponin, flavonoids and organosulfuric compounds have been isolated from *Allium victorialis* var. *platyphyllum*. (Lee and Choi *et al.*, 2001) (Fig. 6) Because this plant has been traditionally known as anti-aging vegetables in Korea, we investigated the cytotoxicity for elucidation of anti-tumour activity. Only gitogenin 3-*O*-lycotetroside (**19**) showed significant cytotoxocities on various cancer cells. The native organosulfuric compounds, S-alkyl- and S-alkenyl cysteines that can be obtained from Allium species (Lawson *et al.*, 1991), expecially *Allium sativum*, exhibit no cytotoxicities probably due to their

Table 4. Effect of Eugenol, a constituent of the *Eugenia caryophyllata* on hepatic drug-metabolizing enzymes

group	normal	eugenol (17)	
		5 mg/kg	10 mg/kg
MDA[2]	$23.9 \pm 0.37^{1)e}$	35.8 ± 0.29^{d}	38.7 ± 0.33^{c}
glutathione *S*-transferase[3]	208.6 ± 11.43^{a}	164.8 ± 15.34^{b}	173.2 ± 13.86^{b}
glutathione[4]	2.17 ± 0.21^{a}	1.68 ± 0.22^{b}	1.60 ± 0.19^{b}
epoxide hydrolase[5]	12.2 ± 0.24^{a}	8.34 ± 0.16^{c}	8.17 ± 0.13^{c}
aminopyrine demethylase[6]	2.84 ± 0.13^{d}	3.37 ± 0.19^{c}	3.78 ± 0.15^{b}
aniline hydroxylase[7]	0.60 ± 0.09^{c}	0.84 ± 0.05^{b}	0.91 ± 0.04^{b}
aldehyde oxidase[8]	1.36 ± 0.09^{c}	2.90 ± 0.10^{b}	$2.98 \pm 0.08^{a,b}$
xanthine oxidase[9]	2.33 ± 0.16^{d}	4.19 ± 0.15^{c}	4.59 ± 0.19^{b}

Rats were intraperitoneally injected daily for seven days and animals were decapitated 24 h after the last injection. [1]Vlaues are mean ± S.D. for six experiments. Values with same superscript letter are not significantly different in each row.($p < 0.05$); Unit: [2]nmol/g of tissue [3]1,2-dinitro-4-nitrobenzene nmol/mg protein/min [4]μmol/g of tissue [5]nmol/g of tissue [6]HCHO nmol/mg protein/min [7]p-aminophenol nmol/mg protein/min [8]2-pyridone nmol/mg protein/min [9]uric acid nmol/mg protein/min.

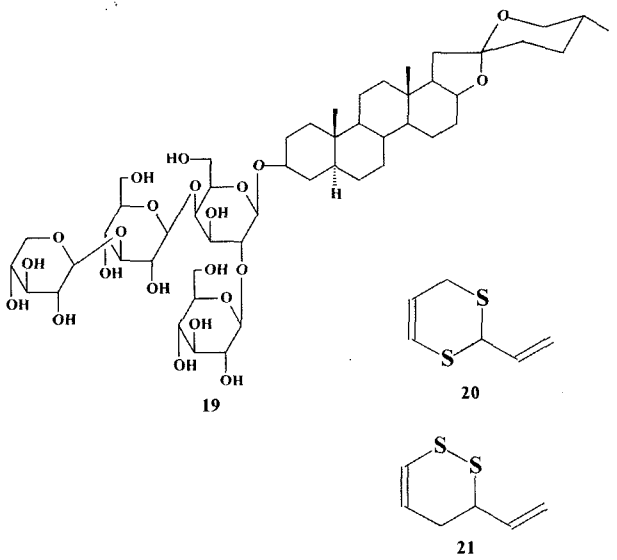


Fig. 6. Structure of steroidal saponin (**19**) and disulfides (**20**, **21**) isolated from *Allium victorialis* var. platyphullum.

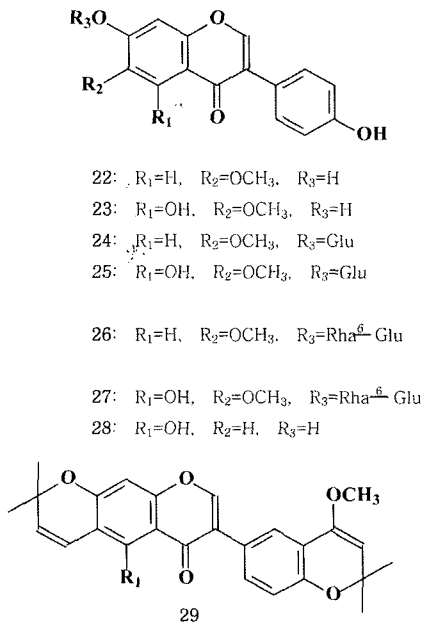


Fig. 7. Structure of isoflavonoids (**22-28**) from *Pueraria thunbergiana*, genistein (**28**), and mutenone (**29**).

high polarities. However, their metabolized products obtainable from the native form are most volatile organosulfuric compounds, disulfides that could show significant cytotoxicities. The major volatile components that obtained from the incubation of the pulverized A. *victorialis* var. *platyphyllum* were identified as 2-vinyl-4H-1,3-dithiin (**20**) and 3,4-dihydro-3-vinyl-1,2-dithiin (**21**) by GC-MS data (Nishimura *et al.*, 1988). The cytotoxicities could be attributable to the exomethylene and the possibility for the binding with intracellular glutathione also could be presumed. Meanwhile, an organosulfuric cysteine derivative, S-allylmercaptocysteine was reported to have hepatoprotective and we could not find the literature on its cytotoxicity (Hikino *et al.*, 1986). It could be pointed out that hydrophilicity is too high to pass through the bilayer of cell membrane. Based on the effectiveness of disulfides for anti-cancer activities, many derivatives have been synthesized by chemical methods (Lee, Lee and Kim *et al.*, 2001).

Isoflavones – We have isolated isoflavonoids from *Pueraria thunbergiana* (Park *et al.*, 1999). Although no cytotoxicity of the isolates, glycitein (**22**), tectorigenin (**23**), glycitin (**24**), tectoridin (**25**), 6"-xylosylglycitin (**26**), 6"-O-xylosyltectoridin (**27**), were shown, the only isolate tectorigenin showed significant cytotoxicity (Bae *et al.*, 1999). (Fig. 7, Table 5) A number of isoflavones was

Table 5. Cytotoxic activity (IC_{50}) of isoflavones (**22-27**) isolated from *P. thunbergiana* on cancer cell growth *in vitro*[a]

cell line	HL-60	U-937	HepG2	SNUC-5
tectorigenin (**23**)	22.33[a]	27.97	83.96	62.65
glycitein (**22**)	26.36	103.5	136.5	165.8
tectoridin (**25**)	> 200	> 200	> 200	> 200
glycitin (**24**)	> 200	> 200	> 200	> 200
6"-*O*-xylosyltectoridin (**27**)	> 200	> 200	> 200	> 200
6"-*O*-xylosylglycitin (**26**)	> 200	> 200	> 200	> 200
genistein	8.54	22.24	39.89	26.65

[a]IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number as compared with that of the control cultures in the absence of inhibitor. The values represent the mean of three independent experiments.

known to have insecticidal activity in addition to the cytotoxicity. The anticancer effect of genistein (**28**) was deeply studied more than of any other isoflavonoids (Kumi-Diaka *et al.*, 2000; Yang *et al.*, 2000). Although genistein was known as an inhibitor of protein tyrosine kinase (Bergan *et al.*, 2001), it might be a typical phenomenon of signal transduction. When we compare the structure of tectorigenin and genistein, we can find that tectorigenin has an additional 6-methoxy than genistein. However, the IC_{50} value of tectorigenin is a little higher than genistein. The cytotoxicity of glycitein without a 5-OH was very weak. All other isoflavone glycosides did not show cytotoxicity (Lee and Sohn *et al.*, 2001). Of course, either cytotoxicity or no cytotoxicity will depend on the structures. Although genistein cannot belong to a quinone class at all, the cytotoxicity of genistein including many other isoflavones may be caused like cytotoxic quinone compounds because both cytotoxic quinones and genistein suffer from the action of quinone reductase (Lee *et al.*, 1999). It is known that quinone reductase produces from quinones to semiquinone radical, which may have an ability to offer oxidative stress (Young *et al.*, 1992). We have no information of the precise role of 4'-OH and 7-OH though they may play important roles for the cytotoxicity including other bioactivities (Wei *et al.*, 1995). It would be possible that we assume the low oxidation-reduction potential of genistein in cells depending on the highly conjugated double bond system and 4'- and 7-OHs. We can also assume the conversion ability of genistein in structure from genistein to a keto-form based on its oxidizable properties. However, we have no exact evidences for our assumption unfortunately. As found in reports on the cytotoxicity of isoflavone glycosides, our own samples also showed no cytotoxicity. Undoubtedly, glycosides linkage at 7-OH may hinder the active moiety of isoflavones so that they are hardly permeable to cell membranes. The research on the biotranformation of 6"-O-glycosyltectoridin by human intestinal bacteria revealed slow hydrolysis rate of the outer rhamnosyl moiety and fast cleavage of the inner glucose (Bae *et al.*, 1999). This research revealed also that the active moiety of the starting compound for the cytotoxicity and hypoglycemic effect should be a produced genin (Bae *et al.*, 1999). It would be a great concerning that cytotoxic isoflavonoids shares other valuable *in vivo* and *in vitro* tests (Bae *et al.*, 1999). Many beneficial bioactivities of isoflavonoids may depend on the inhibition of cyclooxigenase, inducible nitric oxide, tumour-necrosis-factor-α production (Shin *et al.*, 1999) and these formations will be absolutely induced by gene expressions. Understanding on these aspects of isoflavones including genistein and tectorigenin may assist the development of isoflavones as available cancer chemopreventives and other purposes. Based on the assumption that the cytotoxicity may depend partly on the sensitivity of isoflavones to glutathione conjugation, one can design more reasonable experiment for elucidation of mechanism of the bioactivity of isoflavones. Unlike the expectation on specific ligand formation for the activities, it is being believed that the activities of isoflavones do not have to do with such. Since tectorigenin has the activities such as free-radical scavenging (Lee and Sohn *et al.*, 1999) lacking in genistein, inducible nitric oxide synthase inhibitory, PGE_2 formation inhibitory effect (Bae *et al.*, 1999), cytoprotective, the inhibitory of NADPH-induced lipid peroxidation (Park and Jung *et al.*, 2002), (Table 6) antimutagenic (Lee and Sohn *et al.*, 2000) and *in vivo* hypoglycemic (Lee and Sohn *et al.*, 2000), it could be a promising anti-aging agent. Many of them could be induced by the cell-damaging progress associated with oxidative stress. Throughout many reports on the cytotoxicity of isoflavones, it was deduced that genistein could be a basic skeleton for the cytotoxicity of isoflavones. Being dependent on this type of biological

Table 6. IC_{50} values in antioxidant assay of the constituents of *Pueraria thunbergiana* flowers

compound	DPPH[1)]	XOD[2)]	superox. anion[3)]	lipid peroxidation	
				Fe^{2+}/ascorbate[4)]	Fe^{3+}/ADP/NADPH[5)]
glycitein (**22**)	71.0	> 500	44.7	22.9	142.0
tectorigenin (**23**)	94.4	328.4	53.7	23.5	35.0
genistein (**28**)	> 1,000	> 500	292.2	30.9	44.7
glycitin (**24**)	61.0	> 500	> 500	66.8	> 500
tectoridin (**25**)	357.5	180.8	> 500	69.2	174.6
α-tocopherol	3.6	–	–	–	–
allopurinol	–	0.12	–	–	–
caffeic acid	–	–	0.75	0.66	0.84

Each value is the mean of three independent experiments.
[1)]Superscript means DPPH free radical scavenging assay.
[2)]Superscript means xanthine oxidase inhibitory assay.
[3)]Superscript means superoxide scavenging assay.
[4)]Superscript means non-enzymatic (ascorbic acid)-lipid peroxidation inhibitory assay.
[5)]Superscript means enzymatic (NADPH)-lipid peroxidation inhibitory assay.

ability, chemical modification of isoflavone structure may either potentiate or reduce activities. Lee *et al.* (Lee and Song *et al.*, 1999) have reported the availability of genistein as a cancer chemopreventive and the inhibitory activity of mutenone (**29**) on 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced ornithine decarboxylase (Lee and Luyengy *et al.*, 1999).

Flavonoids – Flavonoid is designated as hydrophilic pigments with C_6-C_3-C_6 unit found in natural resources. Flavonoids are found in most parts of plants, and play a major role in antioxidation. Various types of substituent on the basic skeleton flavone will certainly change the bioactivities. Many researchers are mainly concerned in the effect of the substituent OHs of flavonoids rather than in α,β-unsaturated ketone of the central ring, γ-pyrone (Choi *et al.*, 1994). However, we estimate that they did not reach the satisfactory conclusion. The α,β-unsaturated ketone is important for the Michael addition. For the lower activity of flavones with methylated- or with less hydroxylated structure than flavone itself (Hou *et al.*, 1994), it can be generally designated that the hydroxyl substituents should result in the decrease of potency. We also have to consider the possibility to block signal transduction partially due to the antioxidative ability since signal transduction theory have been established that can be mediated by ROS. In addition, *o*-dihydroxyl in B-ring and carbonyl structure elongated from A-ring could be involved in the cytotoxicity of flavanone on HeLa cell (Mori *et al.*, 1988).

Prenylated flavonoids have prenyl chain bearing an exomethylene in which could be an active moiety (Ko *et al.*, 1999; Seo *et al.*, 1997; Kang *et al.*, 2000). (Fig. 8)

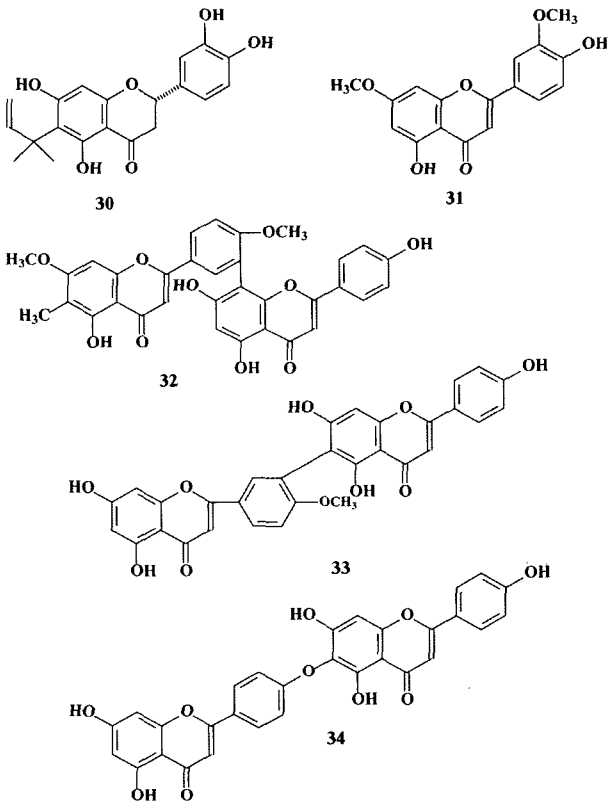
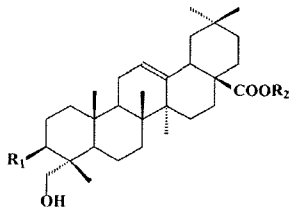


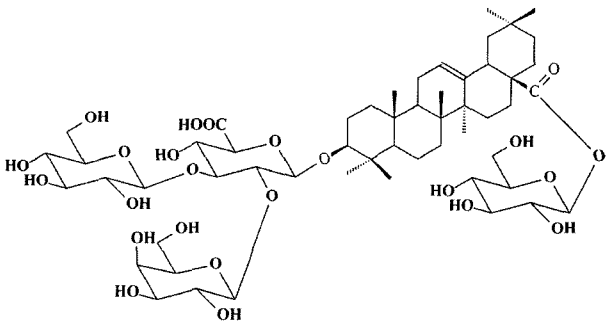
Fig. 8. Cytotoxic monomeric and dimeric flavonoids (**30-34**). {**30** (6,8-diprenyleridictyol), **31** (velutin), **32** (taivanoflavone-A), **33** (robustaflavone-4'-methyl ether), and **34** (hinokiflavone)}

From the cytotoxicity of 6,8-diprenyleridictyol (**30**) the elongated double bond from aromatic ring also could contribute to the cytotoxicity, where the prenyl chain does not contain any exomethylene. Velutin (**31**), a kind of potent cytotoxic flavonoid, with methoxyl but no hydroxyl

groups, may deduce the importance of binding ability with glutathione and subsequent oxidative stress for the cytotoxicity (Zahir *et al.*, 1996). It could be deduced, as a kind of important fact, that the inhibitory action of velutin on topoisomerase I will be crucially caused by the signal transduction started by oxidative stress. Habtemariam (1997) distinguished the flavonoids into the elevating and inhibitory ones on TNF-α dependent cytotoxicity. In our opinion, it was considered that this report led to the conclusive explanation for the antioxidant or prooxiant role for the elevating or inhibitory effect of flavonoids. The potent cytotoxic biflavones (Kuo *et al.*, 2000; Lin *et al.*, 2000; Lin *et al.*, 1989), dimeric flavones (**32-34**), were not revealed in the role of highly conjugated double bond systems.

Triterpenes and tritriterpenoidal saponins – Saponin has been traditionally designated as a group of natural products with surfactant activity. It can be also designated chemically as a group composed of triterpenoid glycosides or steroid glycosides. The stem bark of *Kalopanax pictus* has been used for the tonic, anti-diabetic and anti-rheumatoidal drugs in Oriental medicine (Moon, 1991). During our research on the effectiveness of active principles it was found that the exact action was associated with the cytotoxicity. The components without cytotoxicity did not show any activity in any assay system of us. *K. pictus* contained typical saponin constituents (**35-42**, Fig. 9), hederagenin glycosides which are classified into hederagenin monodesmosdies and bisdesmosides (Sano *et al.*, 1991; Shao *et al.*, 1989). Furthermore, inactive bisdesmosides were transformed to active monodesmosides by human intestinal bacteria (Kim and Yu *et al.*, 1998). Our intensive studies revealed interesting structure-activity relationship and suggested the possibility for the availability of saponins. Hederagenin (**42**) shows mild cytotoxicity, but the IC_{50} of δ-hederin (**38**) with arabinose attachment to hederagenin was not be determined on our various cancer cell lines (Park and Kwon *et al.*, 2001). (Table 7) Linkage of disaccharide moiety to hederagenin plays an important role in the significant cytotoxicity since the second sugar attached to 2'-position of δ-hederin changed from an inactive glycoside to active glycoside (kalopanaxsaponin A, **39**). However, the linkages of disaccharide to hederagenin do not always exhibit cytotoxicity. Mimaki *et al.* (1999) reported no cytotoxicity of 4'-O-glucosyl-δ-hederin (**43**) while other hederagenin monodesmosides showed potent cytotoxicity. On the other hand, the apoptogenic properties of oleanolic acid glycoside bearing the second sugar attachment to 3-OH of the first have reported recently (Ahn *et al.*, 2000). The most unique





44

35 R_1=α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl
R_2=α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside

36 R_1=β-D-xylopyranosyl-(1-3)-α-L-rhamnopyranosyl -(1-2)-α-L-arabinopyranosyl
R_2=α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside

37 R_1=β-D-glucopyranosyl-(1-4)-β-D-xylopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-2)
-α-L-arabinopyranosyl
R_2=α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside

38 R_1=α-L-arabinopyranosyl R_2=H

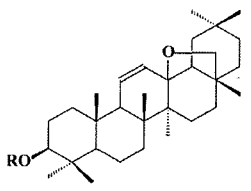
39 R_1=α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl R_2=H

40 R_1=β-D-xylopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl R_2=H

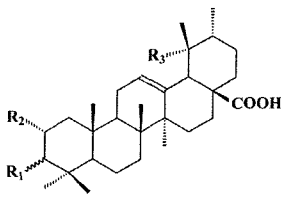
41 R_1=β-D-glucopyranosyl-(1-4)-β-D-xylopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-2)
-α-L-arabinopyranosyl R_2=H

42 R_1=H R_2=H

43 R_1=β-D-glucopyranosyl-(1-4)-α-L-arabinopyranosyl R_2=H



45 : R=Fuc^{3}-Glc-
50 : R=Rha^{2}-Glc^{4}-Ara-
|^{2}
Glc



46 : R_1=β-OH, R_2=H, R_3=H
47 : R_1=O, R_2=H, R_3=H
48 : R_1=β-OH, R_2=H, R_3=H
49 : R_1=β-OH, R_2=OH, R_3=OH

Fig. 9. Structure of saponins (**35-41**) and their aglycone (**42**) isolated from the stem bark of *Kalopanax pictus* and **43** and other triterpenoids (**44-50**)

Table 7. Cytotoxic activities of the constituents of *K. pictus* on tumor cell growth

compound	EC_{50} (μM)[a]			
	J82	T24	Colon26	3LL
hederag. (**42**)	86.4 ± 5.0	81.4 ± 9.8	61.0 ± 8.9	59.3 ± 9.5b
δ-hederin (**38**)	> 166	> 166	> 166	> 166
kalopanaxsap. A (**39**)	15.5 ± 1.1	16.5 ± 1.2	12.5 ± 0.7	10.1 ± 0.5
kalopanaxsap. I (**40**)	6.4 ± 0.5*	5.2 ± 0.9*	2.7 ± 1.1**	2.2 ± 0.2**
sapindoside C (**41**)	4.4 ± 0.9*	6.4 ± 1.2*	1.3 ± 0.2**	1.2 ± 0.3**
cisplatin	14.0 ± 3.6	18.3 ± 4.0	12.0 ± 0.7	11.3 ± 0.7

[a] IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number.
[b] The values represent the mean of three independent experiments.
* $P < 0.05$, ** $P < 0.01$

difference in structure among the glycosides discussed above is axial attachment of the second sugar in 4'-O-glucosyl-δ-hederin. A uronic acid attachment to a triterpene showed cytotoxicity though it is a triterpene monosaccharide (Park *et al.*, 2002). We assess the importance of carboxyl group in uronic acid for the cytotoxicity, which implies the significance of the decrease in electron density of C-3 position. The third sugar attachment enhanced the cytotoxicity of kalopaxsaponin A while the fourth sugar attachment did not enhance the activity of hederagnin trisaccharide. We could assume the possibility for the property on necrosis in addition to on apoptosis, which could be attributed to the resistance of plant cells against fungal cells. In contrast, hederagenin bisdesmosides did not show cytotoxicities. Surfactant represents the compounds with both hydrophilic and hydrophobic moieties capable of emulsifying the two phases into the mixed solution. Since kalopanaxsaponin A has two parts of both hydrophilic sugar moiety and hydrophobic triterpene moiety, obviously it belongs to surfactants. When the structure of space-filling-model of kalopanaxsaponin A was simulated by a computer program, the structure was very interestingly found not to be bending but linear. Hemolytic properties of saponin may be dependent on such a surfactant activity capable of producing severe cell damage. Here, we can presume no cytotoxicity of bisdesmosides together with loss of surfactant activity based on the composition of hydrophilic-hydrophobic-hydrophilic structure. Although bisdesmosyl saponin (**44**) isolated from *Aralia dasyphylla* (Arlaiaceae) was shown to have a significant cytotoxicity (Xiao *et al.*, 1999), the reason could be attributable to not enough sugar attachment to 28-carboxyl. Although it is not an example for the cytotoxicy, there is an interesting report that a saponin with a sugar moiety to 28-position was active in anti-inflammatory test. In addition, a methylated product of 28-carboxyl of triterpene acid maintained hemolysis and anti-complement activity (Park and Oh *et al.*, 1999). From the discussed above it should be understood why three sugars in kalopanxsaponins B (**35**), H (**36**) and K (**37**) are linked. Since there have been revealed a number of saponin structures, successive structure-activity relationship on saponins should be pursued. However, we have little doubt on structure-cytotoxicity relatioship seriously different from our assumption.

We obtained additional evidence on our assumption from the research on apoptosis and signal transduction of saponins. Kalopanaxsaponin A inhibited the expressions of anti-apoptotic *bcl-2* and activated pro-apoptotic protein *c-myc*. (Kim and Park *et al.*, 2002) In addition, mitochondrial cytochrome c release was observed and the activation of caspase-3 led to typical DNA laddering found in apoptosis. Treatment of a chelating agent EDTA or EGTA to the medium led to the loss of apoptogenic ability induced by kalopanaxsaponin A, which suggest that **39** induced Ca^{2+} mediated apoptosis. We could presume the action of **39** on membrane proteins for the Ca^{2+} influx based on the action on integral or peripheral protein of cell membrane. We must consider the susceptibility of biomembrane to amphipathic compounds (Lehninger *et al.*, 1993). The increase of intracellular Ca^{2+} was observed when treated with **39**. The pretreatment of EGTA induced differentiation of cancer cells instead of apoptosis.

From the above discussion the apoptosis induced by **39** could be attributed to Ca^{++}-influx due to the alteration of membrane protein and subsequent signal transduction. Membrane alteration caused by saponins was observed during the research of antileishmanicidic effect (Ridoux *et al.*, 2001; Delmas *et al.*, 2000). Since many saponins including **39** have ionizable structure with big molecular size, it would be hard to penetrate cell membrane. When in most cases considering the structure of triterpeneoid saponin with 28-carboxyl, we could find most of them

were designed to hardly pass through cell membrane. We also expect the future precise research on the ligand formation between a certain cytotoxic saponin and biomolecules such as membrane protein, phosphate of phospholipid and extracellular calcium ion. There is another evidence on the hard traverse to cell membrane that was identified during the research on the absorption of glucose from the intestine (Yoshikawa *et al.*, 1997). It is well known that glucose is absorbed by the glucose-transporter residing in epithelium cell of the intestine (Nelson *et al.*, 1993). As discussed above, the first stage of **39** for the apoptosis may be Ca^{2+}-influx caused by membrane alteration.

Before the establishment of apoptosis induced by **39**, we have observed antimutagenic (Lee, Sohn, and Park *et al.*, 2000), anti-lipid peroxidative (Choi and Huh *et al.*, 2001), anti-hypoglycemic (Park and Kim *et al.*, 1998) and antinociceptive (Choi and Huh *et al.*, 2002) action of **39**. Most of their actions are well associated with ROS level in the body. The active components were identified among hederagenin monodesmosides on every assay system rather than antimutagenic one. However, the possibility for the hydrolysis of hederagenin bisdesmosides could be assumed among hepatic homogenate (S9 mix). (Table 8, 9) There is a report that treatment of α-hederin increased metallothionein in the rat (Iszard *et al.*, 1995). On our experiments, **39** actually decreased the formation of NO, cylooxigenase and TNF-α in LPS-activated macrophage cell (Kim and Park *et al.*, 2002). As discussed above, it should be assessed that the crude drugs containing saponins have been used as anti-inflammatory drugs. Synthetic corticoids used for the anti-inflammatory purposes show apoptosis through the inhibition of *bcl*-2 and p53 and activation of *c-myc* in a high concentration (Kim and Park *et al.*, 2002). However, synthetic corticoids can penetrante nuclear membrane and bind with chromatin

Table 8. Effect of the constituents of *K. pictus* on the mutagenicity induced by aflatoxin B_1 (AFB_1, 1.5 μg/plate) in *Salmonella typhimurium* TA100

treatment(μg/plate)	revertants/plate		
	0.5	2.5	5.0
AFB_1 + **42** (hederagenin)	$831 \pm 13^{de1)}$ (23)[2)]	659 ± 15^{d} (42)	437 ± 11^{d} (67)
39 (kalopanaxsaponin A)	513 ± 22^{f} (59)	474 ± 25^{e} (63)	415 ± 5^{d} (70)
40 (kalopanaxsaponin I)	790 ± 18^{e} (28)	701 ± 7^{c} (38)	636 ± 34^{b} (45)
35 (kalopanaxsaponin B)	852 ± 27^{cd} (21)	776 ± 17^{b} (29)	444 ± 19^{cd} (67)
36 (kalopanaxsaponin H)	860 ± 24^{cd} (20)	797 ± 19^{b} (27)	642 ± 22^{b} (44)
AFB_1 (Control)	1034 ± 47^{a}		
spontaneous	148 ± 8^{2}		

[1] Values represent mean ± S.D. based on three experiments.
[2] The values in parentheses are the inhibition rates (%).
[a~f] Means with the different letters beside symbols are significantly different at the 0.05 level of significance as determined by Duncan's multiple range test.

Table 9. Effect of the constituents of *K. pictus* on the mutagenicity induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 0.3 μg/plate) in *Salmonella typhimurium* TA100

treatment (μg/plate)	revertants/plate		
	0.5	2.5	5.0
MNNG + **42** (hederagenin)	$1294 \pm 11^{1)}$ (–)	1264 ± 15 (3)[2)]	1222 ± 15 (6)
39 (kalopanaxsaponin A)	1362 ± 25 (–)	1337 ± 28 (–)	1263 ± 7 (3)
36 (kalopanaxsaponin I)	1384 ± 19 (–)	1364 ± 9 (–)	1306 ± 9 (–)
35 (kalopanaxsaponin B)	1371 ± 11 (–)	1313 ± 7 (–)	1260 ± 19 (3)
40 (kalopanaxsaponin H)	1382 ± 22 (–)	1379 ± 15 (–)	1344 ± 11 (–)
MNNG (Control)	1294 ± 29		
spontaneous	122 ± 8		

[1)] Values represent mean ± S.D. based on three experiments.
[2)] The values in parentheses are the inhibition rates (%).

unlike saponin. Saikosponin-a (**45**) isolated from *Bupleurum falcatum* has shown similar effects on the signal transduction (Hsu *et al.*, 2000).

We have one more question why the groups or moieties inducing electron deficiency at C-3 increase the potency of apoptogenic ability. As shown in many reports, the more saponins substitute hydroxyl groups at C-2 and –23 neighboring to C-3, the more cytotoxic abilities are found. Cytotoxic and antitumor activities (Min *et al.*, 2000) of ursolic acid (**46**) in addition to the cytotoxic activities of ursonic acid (**47**) (Kim *et al.*, 2000) and 2-hydroxyursolic acid (**48**) (Kim and Ahn *et al.*, 1998) have been reported. These actions will be surely associated with the signal transduction. There is an interesting report that pH changes could alter cytotoxic ability of triterpenes (Noda *et al.*, 1997). We assume that the ionization of 28-carboxyl may be associated with the cytotoxicity of triterpenes. It will be critical for the ionization of carboxyl group to substitute 16- or 22-position. Pomolic acid (**49**) with higher cytotoxicity than ursolic acid actually may be related with the substitution of hydroxyl at C-19 (Neto *et al.*, 2000). A cytotoxic saponin (**50**) reported by Bloor (1994) could be a surfactant composed of hydrophilic-hydrophobic structure.

Oleuropein and phenylethanoid – Iridoid glycosides exist mostly in the form of C-1 glucoside. We can imagine their cytotoxic activity could be shown during the cleavage of glycoside bond. The hydrolysis makes iridoid glycosides equilibrate between hemiacetal- and dial structure (Lee *et al.*, 1995). Oleuropein (**51**) found in olive oil is also a well-known iridoid glycoside that has been assessed as an antioxidant. We have isolated this iridoid glycoside and phenylethanoid glycoside along with lignan glycosides. In our cytotoxicity test, only oleuropein and 3,4-dihydroxyphenethyl alcohol 8-O-glucoside (**52**) showed cytotoxicity (Park *et al.*, 1999). (Fig. 10) The two structures share a 3,4-dihydroxyphenethyl moiety and therefore oleuropein was hydrolyzed in alkaline solution (Table 10). The two isolates from the product that the aromatic moiety has been removed showed no cytotoxicity. We have to note the importance of the active 3,4-dihydroxyphenethyl which can be equilibrated with keto-form. The possibility that oleuropein can be metabolized in certain situation of a living system will exist and could manifest cytotoxicity. In cases of phenylethanoid glycosides, there are many complex forms of glycosides that have oligosaccharides in addition to phenylpropanoid and phenylethanoid. However, it was our good opportunity to discuss the structure-cytotoxicity relationship on phenylethanoid glycosides because these

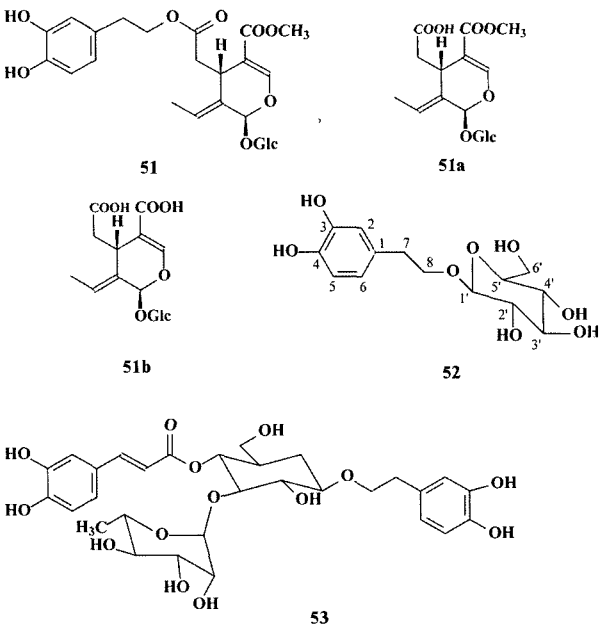


Fig. 10. Structure of oleuropein (**51**), its alkaline hydrolysates (**51a** and **51b**), 3,4-dihydroxyphenylethyl alcohol 8-O-β-D-glucopyranoside (**52**) and verbascoside (**53**).

Table 10. Cytotoxic activities of **51**, **51a**, **51b**, and **52** on tumor cell growth

compound	IC_{50} (μg/ml)[a]			
	P-388	L-1210	SNU-5	HL-60
51	75.5	46.5	13.4	22.5
51a	> 1000	> 1000	> 1000	> 1000
51b	> 1000	> 1000	> 1000	> 1000
52	2.7	2.7	47.9	8.0

[a] IC_{50} is identified as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

almost always have 3,4-dihydroxyphenethyl alcohol 8-O-glucosyl moiety. There is a report on the anti-tumor activity of verbascoside (**53**) also called under the name of acteoside. In this report, it was suggested that verbascoside inhibited protein kinase C binding competitively with catalytic domain of protein kinase C (Herbert *et al.*, 1991). On the other hand, Xiong *et al.* (2000) have reported that acteoside interferes with reactive oxygen intermediates (ROI) for apoptosis-signal transduction, depending on different concentrations and cellular environment.

Quinone – Quinone can be chemically designated as a group of compounds with the structure of cyclohexadienedione. Although there are typical quinones that can be classified as benzoquinone, naphthothoquinone, anthra-

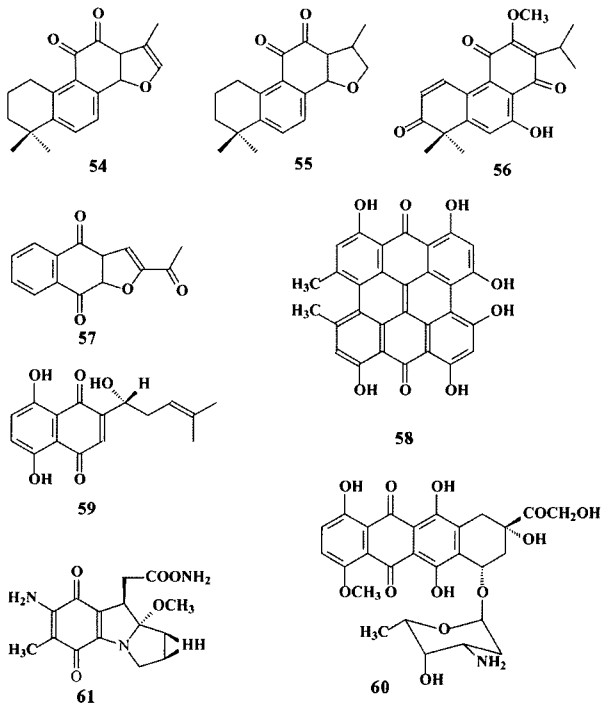


Fig. 11. Structure of natural quinines (**54-61**) with cytotoxicity. {**54** (tanshinone IIA), **55** (dihydrotanshinones IIA), **56** (agastaquinone), **57** (furonaphthoquinone), **58** (hypericin), **59** (shikonin), **60** (adriamycin), **61** (mitomicin C).

quinone, other various types of quinones are also found in natural resources. Additionally, a number of quinones with potent cytotoxicity have been reported. Many diterpene quinone isolated from *Salvia miltiorrhiza* showed mostly potent cytotoxicities (Ryu *et al.*, 1997). (Fig. 11) A little more potent cytotoxicity of tanshione IIA (**54**) than that of dihydrotanshione IIA (**55**) could be attributed to one less number of double bond. It would be important that these diterpene quinones show multi-drug resistant cytotoxicity. Tanshionone IIA, the most abundantly contained one, induced apoptosis toward the pathway of caspase-3 activation (Yoon *et al.*, 1999). Lee *et al.* (1995) have reported that agastaquinone (**56**) from Labiatae family showed a considerably potent cytotoxicity. Since potent cytotoxicities of 1,4-anthraquinones and 1-azaanthraquinones also have been reported (Soonthornchareonnon *et al.*, 1999), the resultant oxidation-reduction potential due to quinone moiety may be involved in apoptosis. As found in the structure of quinones with potent cytotoxicites, e.g., furonapththoquinone, (**57**) it should be mentioned that less number of hydrophilic groups in addition to more extended double bonds involve in the potency of cytotoxicity (Rao *et al.*, 1982). Hypericin (**58**), one of extended quinone compounds, exhibited much potent cytotoxicity together with apoptogenic and differentiaion-inducing ability (Lee and Kim *et al.*, 1999; Kim, Park and Park *et al.*, 1998). This potent effect may be dependent on the highly conjugated double bond system capable of producing a very low redox potential and therefore provide photodynamic toxicity (Delaey *et al.*, 1999). Shikonin (**59**) isolated from *Lithospermum erythrorhizon*, which is belonging to naphthoquinone, was reported to have the inhibitory activity on topoisomerase I and to have apoptosis-inducing activity (Yoon *et al.*, 1999). The cytotoxicity of quinones generally could be caused by the semiquinone radical. Noda *et al.* (1997) divided naphthoquinones into two groups: one is a group that could be attenuated by glutathione and the other is a group that not attenuated by the thiol compound. Since adriamycin (**60**) and mitomicin (**61**), clinically important anticancer drugs of the quinone class were not attenuated by glutathione, the cytotoxicity of a typical cytotoxic quinone may be due to the formation of semiquinone radical, not due to glutathione-conjugation ability.

Cardiac glycosides – Cardiac glycosides could be designated as steroid glycosides with five- or six-membered lactone ring at C-17 causing the contraction of heart muscle. Almost all the aglycone of the cardiac glycosides belongs to cardenolide or bufadienolide. The cardiac action mechanism of those glycosides (**62**, **63**) was known to depend on the inhibitory activity on Na^+-K^+-ATPase (Nelson *et al.*, 1993). (Fig. 12) Meanwhile, the cytotoxic activities of those glycosides were reported to be potent (Hyun *et al.*, 1995; Ankli *et al.*, 2000). We presume that the potent activity could be associated with the direct inhibitory action on Na^+-K^+-ATPase, though there is no report on the relation between the cytotoxicity and that membrane protein. It should be commented that the compounds weakening this electric potential provide the cellular circumstances for apoptosis. Although the apoptosis and signal transduction of cardiac glycosides are precisely revealed, it should be also considered that oxidative stress to the cell due to cell's redox-potential change is a major driving force for apoptosis. It should be reminded that there is no partial structure with strong electrophilicity in the molecule. We cannot also imagine that these compounds have selectivity against cancer cells.

Others – Polyacetylenes (**64**, **65**) found either among many marine organisms or among some typical terrestrial plants show potent cytotoxicity (Lim *et al.*, 1999; Lim *et al.*, 2001). (Fig. 13) We assume that the triple bonds could be bound with intracellular glutathione because of the ability of additive reaction. A triterpene with isomalbaricane (**66**) isolated from a marine organism shows potent

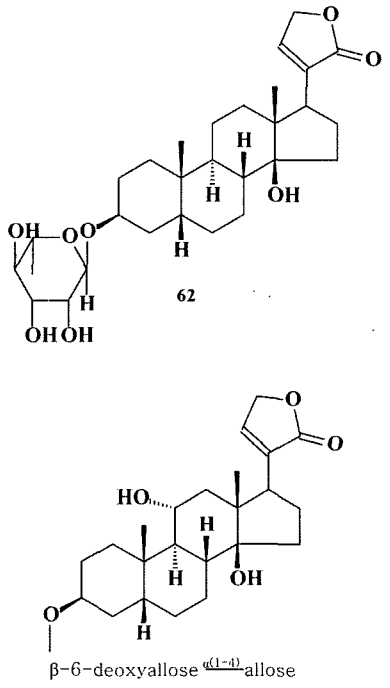


Fig. 12. Cytotoxic cardenolide {**62** (evomonoside), **63**}.

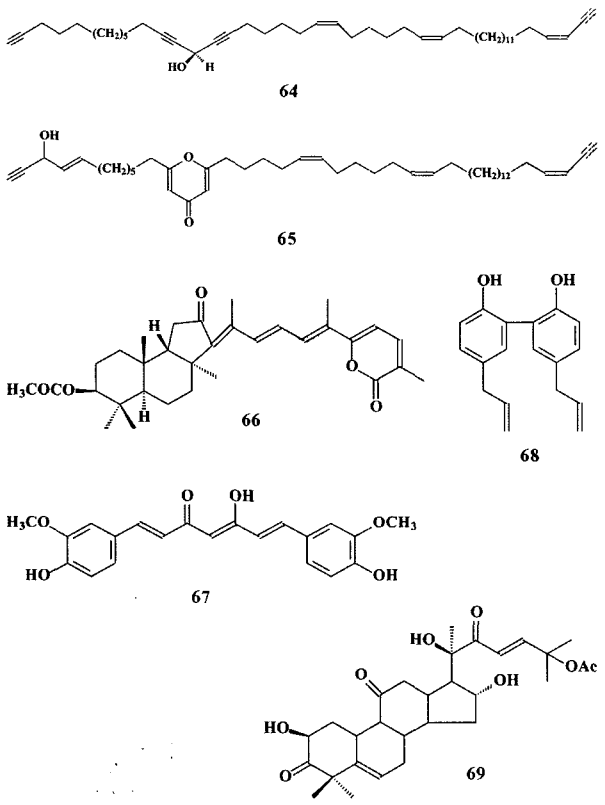


Fig. 13. Structure of cytotoxic natural substwnces (**64-69**) {**67** (curcumin), **68** (magnolol), **69** (cucurbitacin B)}.

cytotoxicity and has a partial structure of conjugated double bonds (Mckee *et al.*, 1997). General diarylhepatanoids (**67**) have a significant cytotoxicity (Syu *et al.*, 1998) and along with the inhibitory activity on cyclooxigenase, inducible nitric oxide synthase (Kim and Ryu *et al.*, 1999). A lignan compound of Magnolol (**68**) with the two exomethylenes has cytotoxicity (Lee *et al.*, 2000). Ryu *et al.* (1994) suggested that α,β-unsaturated ketone of cucurbitacins (**69**) isolated from *Trichosanthes kirilowii* should be an active moiety for the cytotoxicity due to the presumed glutathione-conjugation ability (Ryu *et al.*, 1995).

Conclusion

Cancer is a disease that is difficult to treat because it could be resulted from aging progresses. Therefore, clinical chemotherapy for the malignant tumors may be not simple even for the future. Although a number of researchers have been devoted to the development of anti-cancer drugs from natural products and synthetic chemicals, we have no satisfactory drugs to treat cancer. However, many researchers attempted to develop anti-cancer drugs from natural products. Nevertheless, the cytotoxicity of the compounds can be a clue to solve any biological activity. A number of the research on cytotoxic compounds did not lead to conclusive answer for the development of anti-cancer drugs. In addition, the lack of knowledge on plant chemistry made many researchers lose bioactive compounds.

Plants contain either secondary metabolite that produces oxidative stress on cells and/or others that have antioxidative ability. Some natural compounds, however, have both functions that produce prooxidant and anti-oxidant properties by the same molecules. From this point of view, we could find that the fundamental of life is to preserve biomolecules, especially DNA, from many of oxidative stresses because preservation of genetic substances could inherit to the next generation. When cells meet the oxdative stress, cells inevitably suffer from various biological progresses including mutagenicity and apoptosis. If cells could not react these continued oxidative stresses, individual organism will choose a final selection of death to prevent his species from genetic contamination. Under these assumptions, it has been theoretically hypothesized that the expression of oncogenes may be not occasional occurrence but obviously programmed one.

During the discussion on the cytotoxic natural products we discussed that cells in human body resist oxidative stress. When the substances with oxidative stress were taken to human body, hepatic antioxidant enzymes

including both hepatic microsomal- and cytosolic enzymes will be released immediately. In addition, human bodies are continuously exposed to foreign antioxidant and/or prooxidant materials by oral or other pathways. It should be also noted that some natural compounds could be converted to very electrophilic carcinogen by hepatic actions. Interestingly, Oriental herbal drugs that could be classified into tonic drugs mostly interfere with oxidative stress.

During our research on the active principle of herbal medicine, many active principles were found among the substances with mild cytotoxicity. These substances mostly inhibited the oxidative stress by elevating antioxidant resistance of experimental animals. It was suggested that these compounds could be involved in anti-inflammatory system in the body and these actions may be closely associated with gene expression. In other words, most anti-inflammatory mechanisms are closely associated with so-called anti-lipid peroxidation. These explanations indicate that many herbal drugs could be cancer chemopreventives.

Acknowledgements

This work was supported by a grant (Code # 20050401-034-695-183-01-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

References

Andreasen, M.F., Landbo, A.K., Christensen, L.P., and Hansen, A.A, Antioxidant effects of phenolic rye cereale extracts, monoeric hydroxycinnamates, and ferulic dehydrodimers on human low-density lipoproteins. *J. Agric. Food. Chem.*, **49**, 4090-4096 (2001).

Ankli, A., Heimann, J., Heinrich, M., and Sticher, O., Cytotoxic cardenolides and antibacterial terpenoids from *Crossopetalum gaumeri*. *Phytochemistry*, **54**, 531-537 (2000).

Ahn, K.S., Kim, J.H., Kwon, O.K., Park, S.H., Oh, S.R., and Lee, H.K., Triterpenes and oleanolic acid glycosides isolated from *Tiarella polyphylla* with apoptotic activities on HL-60 clone15 cells. The 31th Annual Meeting of the Korean Society of Pharmacognosy, 52-52 (2000).

Azizan, A. and Blevins, R.D., Mutagenicity testing of six chemicals associated with the pungent properties of specific sp. revealed by the Ames Salmonella/microsomal assay. *Arch. Environ. Contam. Toxicol.*, **28**, 248-258 (1995).

Bae, E.A., Han, M.J., Lee, K.T., Choi, J.W., Park, H.J., and Kim, D.H., Metabolism of 6"-xylosyltectoridin and tectoridin by human intestinal bacteria and their hypoglycemic and *in vitro* cytotoxic activities. *Biol. Pharm. Bull.*, **22**, 1314-1318 (1999).

Bae, E.A., Han, M.J., Lee, K.T., Choi, J.W., Park, H.J., and Kim, D.H., Metabolism of 6"-xylosyltectoridin and tectoridin by human intestinal bacteria and their hypoglycemic and *in vitro* cytotoxic activities. *Biol. Pharm. Bull.*, **22**, 1314-1318 (1999).

Bang, K.H., Mode of Action and Structure-Activity Relationship of Antifungal Trans-Cinnamaldehyde. Thesis for Doctor Degree. Chungnam University, 2000.

Bang, K.H., Lee, D.W., Park, H.M., and Rhee, Y.H., Inhibition of Fungal Cell Wall Synthesizing enzymes by trans-cinnamaldehyde, *Bioscience of Biotechnology and Biochemistry*, **64**, 1061-1063 (2000).

Bergan, R.C., Waggle, D.H., Carter, S.K., Horak, I., and Slichenmyer, W.M., Tyrosine kinase inhibitors and signal transduction modulators: Rationale, current status as chemopreventive agents for prostate cancer. *Urology*, **57**, 77-80 (2001).

Bloor. S., Cytotoxic saponins from newzealand from nyrsine species. *J. Nat. Prod.*, **57**, 1354-1360 (1994).

Chen, Y.J., Shiao, M.S., and Wang, S.Y., The Antioxidant Caffeic acid Phenethyl Ester Induces Apoptosis asociated with Selective Scavenging of Hydroperoxide in Human Leukemic HL-60 Cells. *Anticancer Drugs*, **12**, 143-149 (2001).

Choi, J.H., Seo, B.L., Park, H.J., Lee, J.Y, Lee, Y.S., Park, H.J., Kwon, S.H., and Lee, K.T., Exomethylene group of costunolide induces intracellular thiol depletion and is essential for apoptosis, The Spring Convention of The pharmaceutical society of Korea, PC1-10 (2001).

Choi, J.S., Park, K.Y., Moon, S.H., Rhee, S.H., and Young, H.S., Antimutagenic effect of plant flavonoids in the salmonella assay system. *Arch. Pharm. Res.*, **17**, 71-75 (1994).

Choi, J.W., Huh, K., Kim, S.H., Lee, K.T., Park, H.J., and Han, Y.N., antinociceptive and anti-rheumatoidal effects of *kalopanax pictus* extract and its saponin components in experimental animals. *J. Ethnopharmcol.*, **94**, 199-204 (2002).

Choi, J.W., Lee, K.T., Ka, H., Jung, W.T., Jung H.J., and Park, H.J., Constituents of the Essential Oil of the *Cinnamomum cassia* Stem Bark and the Biological Properties, *Arch. Pharm. Res.*, **24**, 418-423 (2001).

Choi, J.W., Huh, K., Kim, S.H., Lee, K.T., Kwon, S.H., and Park, H.J., Toxicology of *Kalopanax pictus* extract and hematological effect of the isolated anti-rheumatoidal kalopanaxsaponin a on the feund°Øs complete adjuvant reagent-treated Rat. *Arch. Pharm. Res.*, **24**, 119-125 (2001).

Cui, B., Lee, Y.H., Chai, H., Tucker, J., and Fairchild, C., Cytotoxic Sesquiterpenoids from *Columnifera*. *J. Nat. Prod.*, **62**, 1545-1550 (1999).

Delmas, F., Giorgio, C., Elias, R., Gasquet, M., Azas, N., Mshvildadze, V., Dekanosidze, G, Kemertelidze, E., and Tino-David, P., Antileishmanial activity of three saponins isolated from α-hederin, β-hederin and hederacolchiside a compared to their action on mammalian cells culture vitro. *Planta Med.*, **66**, 343-347 (2000).

Delaey, E.M., Kamuhabwa, A.R., Vandenbogaerde, A.L., and de Witte, P.A., Photocytotoxicity of protohypericin after photoconversion to hypericin. *Planta Med.*, **65**, 719-722 (1999).

Fiorio, R. and Bronzetti, A., Effects of cinnamaldehyde on suvival and formatin HGPRT-mutants in V79 cells treated with methyl methanesulfonate, N-nitroso-N-metylurea, ethyl methanesulfonate and UV Light. *Mutation Research*, **324**, 51-57 (1994).

Fujita, T., Takeda, Y., Han, D.S., Minami, Y., Marunaka, T., Takeda, S., Yamada, Y., and Togo,T., Cytotoxic and antitumor activities of rabdosia diterpenoids. *Planta Med.*, **54**, 414-417 (1988).

Habtemariam. S., Flavonoids as inhibitors or enhancers of the Cytotoxicity of tumor necrosis factor-α in L-929 tumor cells. *J. Nat. Prod.*, **60**, 775-778 (1997).

Han D.S., Saengyakhak, Dongmyungsa, Seoul, pp. 402-410, 1992.

Hayashi, T., Nakano, T., Kozuka, M., McPhail, d., Mcphail, a., and Lee, K.H., Antitumor agents. 190. absolute stereochemistry of the cytotoxic germacranolides, tomenphantins a and b, from elephantopus tomentosus. *J. Nat. Prod.*, **62**, 302-304 (1999).

Herbert, J.M., Mafferand, J.P., Taoubi, K., Augereau, J.M., Fouraste, I., and Gleye, J., Verbascoside isolated from lantana camara, an inhibitor of protein kinase C. *J. Nat. Prod.*, **54**, 1595-1600 (1991).

Hikino, H., Tohkin, M., Kiso, Y., Namiki, T., Nishimura, S., and Takeyama, K., Antihepatotoxic actions of *Allium sativum* bulbs. *Planta Med.*, **51**, 163-168 (1986).

Hou, R.S., Duh, C.Y., Wang, S.K., and Chang, T.T., Cytotoxic flavonoids from the leaves of *melicope triphyla*. *Phtychemistry*, **35**, 271-272 (1994).

Hsu, M.J., Cheng, J.S., and Huang, H.C., Effect of sakosaponin, a triterpene saponin, on apoptosis in lymphocytes: association with C-myc, P53 and Bcl-2 Mrna. *Br. J. Pharmacol.*, **131**, 1285-1293 (2000).

Hyun, J.W., Shin, J.E., Lim, K.W., Sung, M.S., Park, J.W., Yu, J.H., Kim, B.K., Park, W.H., Kang, S.S., and Park, J.K., Evomonoside: the Cytotoxic xardiac glycoside from *lepidium apetalum*. *Planta Med.*, **61**, 294-195 (1995).

Iszard, M.B., Liu, J., and Klaasen C.D., Effect of several metallothionein inducers on oxidative stree defense mechanisms in rats. *Toxicology*, **15**, 25-33 (1995).

Ka, H., Choi, J.H., Park, H.J., Han, H.N., and Lee, K.T., Induction of Apoptosis in HL-60 Cells by Cinnamaldehyde Isolated from the Stem Bark of *Cinnamomum cassia* through Depletion of Intracellular Glutathione. The spring convention of the pharmaceutical society of Korea, PC1-6, 2001.

Kang, T.H., Jeong, S.J., Ko, W.G., Kim, N.Y., Lee, B.H., Inagaki, M., Miyamoto, T., Higuchi, R., and Kim, Y.C., Cytotoxic llavadulyl flavanones from *sophora flavescens*. *J. Nat. Prod.*, **63**, 680-681 (2000).

Kim, D.H., Yu, K.W., Bae, E.A., Park, H.J., and Choi, J.W., Metabolism of kaopanaxsaponin B and H by human intestinal bacteria and antidiabetic activity of their metabolites. *Biol. Pharm. Bull.*, **21**, 360-365 (1998).

Kim, J.I., Park, J.H., Park, H.J., Choi, S.K., and Lee, K.T., Induction of differentiation of the human histocytic lymphoma cell line U-937 by hypericin. *Arch. Pharm. Res.*, **21**, 41-45 (1998).

Kim, J.L., Kim H.J., Park, H.J., and Lee, K.T., Mechanism of costunolide-induced differentiation in U937. *Bull. K. H. Pharm.*, **25**, 71-74 (1997).

Kim, Y.K., Park, S.J., Ha, J.H., Choi, J.W., Park, H.J., and Lee, K.T., *In vitro* antiinflammatory activity of kalopanaxsaponin A isolated from *Kalopanax pictus* in murine macrophage RAW 264.7 cells. *Biol. Pharm. Bull.*, **25**, 472-476 (2002).

Kim, S.H., Ahn, B.Z., and Ryu, S.Y., Antitumor effects of ursolic acid isolated from *oldenlandia diffusa*. *Phtytherapy Res.*, **12**, 553-556 (1998).

Kim, Y.K. and Ryu, S.Y., Cytotoxic components from stem bark of *magnolia obovata*. *Planta Med.*, **65**, 291-292 (1999).

Kim, Y.K., Yoon, S.K., and Ryu, S.Y., Cytotoxic triterpenes from stem bark of *physocarpus intermedius*. *Planta Med.*, **66**, 485-486 (2000).

Ko, H.H., Yen, M.H., Wu, R.R., Won, S.J., and Lin, C.N., Cytototoxic isoprenylated flavans of *Broussonetia kazinoki*. *J. Nat. Prod.*, **62**, 164-166 (1999).

Kumi-Diaka, J. and Butler, A., Caspase-3 protease activation during the process of genistein-induced apoptosis in TM4 testicular cells. *Biol. Cell*, **92**, 115-124 (2000).

Kuo, Y.H., Lin, C.H., Hwang, S.Y., Shen, Y.C., Lee, Y.L., and Li, S.Y., A Novel cytotoxic c-methylated biflavone from the stem bark of *Cephalotaxus wilsoniana*. *Chem. Pharm. Bull.*, **48**, 440-441 (2000).

Kwon, B.M., Lee, S.H., Choi, S.U., Park, S.H., Lee, C.O., Cho, Y.K., Sung, N.D., and Bok, S.H., Synthesis and *in vitro* cytotoxicity of cinnamaldehyde to human solid tumor cells. *Arch. Pharm. Res.*, **21**, 147-153 (1998).

Lee, C.W., Hong, D.H., Han, S.B., Park, S.Y., Kim, H.K., and Kwon, B.M., Inhibition of Human Tumor Cell Growth by 2'-hydroxy- and 2'-bezoyloxycinnamaldehyde, *Planta Med.*, **65**, 263-266 (1999).

Lawson, L.D., Wang, Z.J., and Hughes, B.G, γ-Glutamyl-S-alkylcysteines in garlic and other allium spp.: Precursors of age-dependent trans-1-propenylthiosulfinates. *J. Nat. Prod.*, **54**, 436-444 (1991).

Lee H.J., Lee, K.T., Park, H.J., Chi, S.G, and Park, J.H., Costunolide induces mitochodrial cytochrome c release through ROS generation. The spring convention of the pharmeceutical society of Korea, PC1-4 (2001).

Lee, H.K., Oh. S.R., and Lim. J.I., Agastaquinone, a new cytotoxic diterpenoid quinone from Agastache rugosa. *J. Nat. Prod.* **58**, 1718-1721 (1995).

Lee, J.W., Lee, B., and Kim, N.D., Synthesis of 2-(allylthio)pyrazines as a novel cancer chemopreventive agent. *Arch. Pharm. Res.*, **24**, 16-20 (2001).

Lee, K.T., Choi, J.H., Kim, D.H., Son, K.H., Kim, W.B., Kwon, S.H., and Park, H.J., Constituents and the antitumor principle of *Allium victorialis* var. *platyphyllum*. *Arch. Pharm. Res.*, **24**, 44-50 (2001).

Lee, K.T., Kim, J.I., Rho, Y.S., Chang, S.G., Jung, J.C., Park, J.H., and Miyamoto, K., Hypericin induces both differentiation and apoptosis in human promyelocytic leukemia HL-60 cells. *Biol. Pharm. Bull.*, **22**, 1271-1274 (1999).

Lee, K.T., Koo, S.J., Jeong, S.H., Kwon, S.H., and Park, H.J., Structures of three new terpenoids, spiciformisins a and b, and monocyclosqualene, isolated from the herbs of *Ligularia fischeri* var. *spiciformis* and cytotoxicity, The Spring Convention of Korea, PD2-19 (2001).

Lee, K.T., Sohn, I.C., Kim, Y.K., Choi, J.H., Choi, J.W., Park, H.J., and Miyamoto, K., Tectorigenin, an isoflavone of *pueraria thunbergiana*, induces differentiation and apoptosis on human promyelocytic leukemia cells. *Biol. Pharm. Bull.*, **24**, 1117-1121 (2001).

Lee, K.T., Sohn I.C., Kong, E.A., Kim, D.H., Choi, S.K., Choi, J.W., and Park, H.J., Antioxidative and cytoprotective effects of isoflavones isolated from *Pueraria thunbergiana flowers*. *Yakhak Hoeji*, **43**, 736-742 (1999).

Lee, K.T., Sohn, I.C., Kim, D.H., Choi, J.W., Kwon, S.H., and Park, H.J., Hypoglycemic and hypolipidemic effects of tectorigenin and kaikasaponin in the streptozotocin-induced diabetic rat and their antioxidant activity *in vitro*, *Arch. Pharm. Res.*, **23**, 461-466 (2000).

Lee, K.T., Sohn, I.C., Park, H.J., Kim, D.W., Jung, G.O., and Park, K.Y., Essential moiety for antimutagenic and cytotoxic activity of hederagenin monodesmosides isolated from the stem bark of *Kalopanax pictus*, *Planta Med.*, **66**, 329-332 (2000).

Lee, M.W., Kim, J.H., Jeong, D.W., Ahn, K.H., Toh, S.H., and Surh, Y.J., Inhibition of cyclooxigenase-2 expression by diarylheptanoids from the bark of *Alnus hirsuta* var *sibirica*. *Biol. Pharm. Bull.*, **23**, 517-518 (2000).

Lee, M.W., Kim, N.Y., Park. M.S., Ahn, K.H., Toh, S.H., Hahn, D.R., Kim, Y.C., and Chung, H.T., Diarylahepatnoids with *in vitro* inducible nitric oxide activity from *Alnus hirsuta*. *Planta Med.*, **66**, 551-553 (2000).

Lee, S.K., Luyengy, L., Gerhauser, C., Mar, W., Lee, K., Mehta, R.G, Kinghorn, D., and Pezzuto J.M., Inhibitory effect of muneone, an isoflavonoid, on 12-O-tetradecanoylphorbol 13-acetate-induced ornithine decarboxylase Activity. *Cancer Letters*, **136**, 59-65 (1999).

Lee, S.K., Song, L., Mata-Greenwood, E., Kelloff, G.J., Steele V., and Pezzuto, J.M., Modulation of *in vitro* biomarkers of the carcinogenic process by chemopreventive agents. *Anticancer res.*, **19**, 35-44 (1999).

Lee S.Y., Chemistry of Organic Natural Products, Younglimsa, Seoul, 1995, pp. 97-98

Lim, Y.J., Kim, J.S., Im, K.S., Jung, J.H., Lee, C.O., Hong, J., and Kim, D.K., New cytotoxic polyacetylenes from the marine sponge petrosia. *J. Nat. Prod.*, **62**, 1215-1217 (1999).

Lim, Y.J., Park, H.S., Im, K.S., Hong, J.K., Lee, M.Y., Kim, D.K., and Jung, J.H., Additional cytotoxic polyacetylenes from the marines-ponge petrosia species. *J. Nat. Prod.*, **64**, 46-53 (2001).

Lin, L.C., Kuo, Y.C., and Chou, C.J., Cytotoxic biflavonoids from selaginella delicatula. *J. Nat. Prod.*, **63**, 627-630 (2000).

Lin, Y.H., Chen, F.C., and Lee, K.H., Hinokiflavone, A Cytotoxic

principle from *Rhus succedanea* and the cytotoxicity of the related biflavonoids. *Planta Med.*, **55**, 166-168 (1989).

Matsuura, H., Ushiroguchi, T., Itakura, Y., and Fuwa, T., Further studies on steroidal glycosides from bulbs, roots and leaves of *Allium sativum*. *Chem,. Pharm. Bull.*, **37**, 2741-2743 (1989).

McKee, T., Bokesch, H., McCormick, J., Rashid, M., Spielvogel, D., Gustafson, K., Alavanja, M., Cardellina, J., and Boyd, M., Isolation and charaterization of new anti-HIV and cytototoxic leads from plants, marine and microbial organisms. *J. Nat. Prod.*, **60**, 431-438 (1997).

Moon, K.S., Components and utilization of medicinal plants. Ilweolseogak, Pyongyang, pp. 419, 1991.

Mori, A., Nishino, C., Enoki, N., and Tawata, S., Cytotoxicity of pant flavonoids against heLa cells. *Phytochemistry*, **27**, 1017-1020 (1988).

Mimaki, Y., Kuroda, M., Asano, T., and Sashida, Y., Triterpene saponins and lignans from the roots of *Pulsatilla chinensis* and their cytotoxic aactivity against HL-60 Cells. *J. Nat. Prod.*, **62**, 1279-1283 (1999).

Min, B.S., Kim, Y.H., Lee, S.M., Jung, H.J., Lee, J.S., Na, M.K., Lee, J.O., Lee, J.P., and Bae, K.H., Cytotoxic triterpenes from *craetaegus pinnaifida, Arch. Pharm. Res.*, **23**, 155-158 (2000).

Neto, C., Vaisberg, A., Zhou, B.N., Kingston D., and Hammond G., Cytotoxic triterpene acids from the peruvian medidinal plant *Polylepis racemosa. Planta Med.*, **66**, 483-484 (2000).

Nishimura, H., Wijaya, C.H., and Mizutani, J., Volatile flavor components and antithrombotic agents: Vinyldithiins from *llium victorialis. J. Agric. Food. Chem.*, **36**, 563-566 (1988).

Noda. Y., Kaiya, T., and Kohda, K., Enhanced cytotoxicity of some triterpenes toward leukemia L1210 cells cultured in low pH media: possibility of a new mode of cell killing. *Chem. Pharm. Bull.*, **45**, 1665-1670 (1997).

Noda, Y., Kawazoe, Y., and Hakura, A., Cytotoxicity of naphthaquinones toward cultured resting murine leukemia L1210 cells in the presence of glutathione, diethyl maleate, or iodoacetamide. *Biol. Pharm. Bull.*, **20**, 1250-1256 (1997).

Nelson, D.L. and Michael, M.C., Lehninger Principles of Biochemistry 3rd Edition, Worth, New York, pp. 389-489, 1993.

Nelson, D.L. and Michael, M.C., Lehninger principles of biochemistry 3rd edition, Ed.; Ryan M., Press: Worth, NY, pp. 389-489, 1993.

Park, H.J., Lee, M.S., Lee, K.T., Sohn, I.C., and Han, Y.N., Studies on constituents with cytotoxic activity from the stem bark of *syringa velutina. Chem. Pharm. Bull.*, **47**, 1029-1031 (1999).

Park, H.J., Kim, D.H., Choi, J.W., Park, J.H., and Han, Y.N., A Potent anti-diabetic agent from *Kalopanax pictus*, *Arch. Pharm Res.*, **21**, 24-29 (1998).

Park, H.J., Kwon, S.H., Han, Y.N., Choi, J.W., Miyamoto, K., Lee, S.H., and Lee, K.T., Apoptosis-inducing costunolide and a novel acyclic monoterpene from the stem bark of *Magnolia sieboldii. Arch. Pharm. Res.*, **24**, 342-348 (2001).

Park, H.J., Kwon, S.H., Lee, J.H., Lee, K.H., Miyamoto, K., and Lee K.T., Kalopanaxsaponin A is a basic saponin structure for the anti-tumor activity of hederagenin monodesmosides. *Planta Med.*, **67**, 118-121 (2001).

Park, H.J., Kwon, S.H., Yoo, K.O., Sohn I.C., Lee, K.T., and Lee, H.K., Sesquiterpenes from the leaves of *Ligularia fischeri* var. *spiciformis. Planta Med.*, **66**, 783-784 (2000).

Park, H.J., Kwon, S.H., Han, Y.N., Choi, J.W., Miyamoto, K., Lee, S.H., and Lee, K.T., Apoptosis-inducing costunolide and a novel acyclic monoterpene from the stem bark of *Magnolia sieboldii, Arch. Pharm. Res.*, **24**, 342-348 (2001).

Park, H.J., Nam, J.H., Jung, H.J., Ko, C.D., Lee, K.T., and Jung, W.T., Structure of codonoposide isolated from *codonopsis lanceolata* roots and the cytotoxicity of prosapogenins. *J. Agric. Food Chem.*, **50**, 4190-4193 (2002).

Park, H.J., Park, H.S., Jung, W.T., Choi, J.W., and Lee, K.T., Toxicological Aspects of Eugenol Isolated from the Essential Oil of *Eugenia caryophyllata*. The Spring Convention of Korea, PD2-42 (2001).

Park, H.J., Park, J.H., Moon, J.O., Lee, K.T., Jung, W.T., Oh, S.R., and Lee, H.K., Isoflavone glycosides from the flowers of *Pueraria thunbergiana. Phytochemistry*, **51**, 147-151 (1999).

Park, K.Y., Jung, K.O., Choi, J.W., Lee, K.T., and Park, H.J., Kaikasaponin III, a potent antimutagenic saponin, isolated from the flower of *Pueraria thunbergiana. Arch. Pharm. Res.*, **25**, 320-324 (2002).

Park, S.H., Oh, S.R., Jung, K.Y., Lee, I.S., Ahn, K.S., Kim, J.G., and Lee, J.J., Anticomplement activities of oleanolic acid monodesmosides and bisdesmosides isolated from *Tiarella polyphylla. Arch. Pharm. Res.*, **22**, 428-431 (1999).

Rao, M.M. and Kingston, D.G.I., Plant anticancer agents. XII. isolation and structure elucidation of new cytotoxic quinines from *tabebuia cassinoides. J. Nat. Prod.*, **45**, 600-604 (1992).

Ridoux, O., Di, G.C., Delmas, F., Elias, R., Mshvildadze V., Dekanosidze, G., Kemertelidze, E., and Balansard, G., *In vitro* Antileishmanial activity of three saponins isolated from ivy, α-hederin, β-hederin and hederacolchiside A (1), in association with pentamidine and amphotericin B. *Phytotherapy Res.*, **15**, 298-301 (2001).

Ryu, S.Y., Lee, C.O., and Choi. S.U., *In vitro* cytotoxicity of tanshinones from *salvia miltiorrhiza. Planta Med.*, **63**, 339-342 (1997).

Ryu, S.Y., Lee, S.H., Choi, S.U., Lee, C.O., No, Z., and Ahn, J.W., antitumor activity of *trichosanthes kirilowii, Arch. Pharm. Res.*, **17**, 348-353 (1994).

Ryu, S.Y., Choi, S.U., Lee, S.H., Lee, C.O., No, Z., and Ahn, J.W., Cytotoxicity of cucurbitacins *in vitro. Arch. Pharm. Res.*, **18**, 60-61 (1995).

Sano, K., Sanada, S., Ida, Y., and Shoji, J., Studies on the constituents of the stem bark of *Kalopanax pictus. Chem. Pharm. Bull.*, **39**, 865-870 (1991).

Seo, E.K., Silva, G.L., Chai, H.B., Farnswoth, N.R., Cordell, G.A., Pezzuto, J., and Kinghorn, A., Cytotoxic prenylated flavanones from *Monotes engleri. Phytochemistry*, **45**, 509-515 (1997).

Singer, B., The chemical effects of nucleic acid alkylation and their relation to mutagenesis and carcinogenesis. Prog. Nucleic Acid Res. *Mol. Biol.*, **15**, 219-284 (1975).

Shao, C.J., Kasai, R., Xu, J.D., and Tanaka, O., Saponins from Roots of *kalopanax septemlobus* (Yhunb.) koidz., ciqiu: structures of kalopanaxsaponins Ia, Ib and Ic. *Chem. Pharm. Bull.*, **37**, 311-314 (1989).

Shin, K.H., Kim, Y.P., Lim, S.S., Lee, S., Ryu, N., Yamada, M., and Ohuchi, K., Inhibition of prostaglandin E_2 production by the soflavones tectorigenin and tectoridin isolated from the rhizome *Belamcanda chinensis. Planta Med.*, **65**, 776-777 (1999).

Soonthornchareonnon, N., Suwanborirux, K., Bavovada, R., Patarapanich, C., and Cassady, J., New cytotoxic 1-azaanthraquinones and 3-aminonaphthoquinone from the stem bark of *goniothalamus marcanii, J. Nat. Prod.*, **62**, 1390-1394 (1999).

Syu, W.J., Shen, C.C., Don, M.J., Ou, J.C., Lee, G.H., and Sun,C.M., Cytotoxicity of curcumonoids and some novel compounds from *Curcuma zedoaria. J. Nat. Prod.*, **61**, 1531-1534 (1998).

Van lerzel, M.L., Ploemen, J.P., Lo Bello, M., Federici, G., and Van Bla, P.J., Interactions of α,β-unsaturated aldehyde and ketones with human gluthathione S-transferase P1-1. *Chem. Biol. Interact.*, **108**, 67-78 (1997).

Wei, H., Bowen, R., Cai, Q., Barnes, S., and Wang, Y., Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc. Soc. Exp. Biol Med.*, **208**, 124-1128 (1995).

Xiao, K., Yi, Y.H., Wang, Z.Z., Tang, H.F., Li, Y.Q., and Lin, H.W.A., Cytotoxic triterpene saponin from the root bark of *aralia dasyphylla. J. Nat. Prod.*, **62**, 1030-1032 (1999).

Xiong, Q., Tezuka, Y., Kaneko, T., Li, H., Tran, L.Q., Hase, K., Namba, T., and Kadota, S., Inhibition of nitric oxide by phenylethanoid in activated macrophages. *Eur. J. Pharm.*, **400**, 137-144 (2000).

Yang, J., Nakagawa, H., Tsuta, K., and Tsubura, A., Influence of perinatal genistein exposure on the development of MNU-induced mammary carcinoma female sprague-dawley rats. *Cancer Letters*, **149**, 171-179 (2000).

Young, H.S., Kim M.S., Chung, H.Y., Choi, J.S., and Kim B.W., Separation of active chemical components components against sarcoma 180 from stem bark of *Catalpa ovata. J. Korean Cancer Assoc.*, **24**, 167-172 (1992).

Yoon, Y.S., Kim, Y.O., Joen, W.K., Park, H.J., and Sung, H.J., Tanshinoe IIA, an Ingredient of *salvia miltiorrhiza* induced apoptosis in HL60 human premyelocytic leukemia cell line. *J. Ethnopharmcol.*, 121-127 (1999).

Yoon, Y., Kim, Y.O., Lim, N.Y., Jeon, W.K., and Sung, H.J., Shikonin, An Ingredient of lithospermum erythrorhizon induced apoptosis in HL60 human premyelocytic leukemia cell line. *Planta Med.*, **65**, 532-535 (1999).

Yoshikawa, M., Shimada, H., Morikawa, T., Yoshizumi, S., Natsumura, N., Murakami, T., Matsuda, H., Hori, K., and Yamahara, J., Meicinal foodstuffs. VII. On the Saponin constituents with glucose and alcohol absorption-inhibitory activity from a food garnish "Tonuri", the fruit of Japanese *Kochia scoparia* (L.) schard.: Structures of scoparianosides A, B, and C. *Chem. Pharm. Bull.*, **45**, 1300-1305 (1997).

Yusuf, A.H., Apoptosis and the dilemma of cancer chemotherapy. *Blood*, **89**, 1845-1853 (1997).

Zahir, A., Jossang, A., and Bodo, B., DNA Topoisomerase I inhibitors: Cytotoxic flavones from *Lethedon tannaensis. J. Nat. Prod.*, **59**, 701-703 (1996).

Zhang, D., Haruna, M., McPhail, A., and Lee, K.H., Cytotoxic ermacranolides of *Elephantopus Carolinianus* and the structure and stereochemistry of isodeoxyelephantopin. *Phytochemistry*, **25**, 899-904 (1986).

(Accepted November 19, 2006)