We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Functional Analysis of Natural Polyphenols and Saponins as Alternative Medicines

Hiroshi Sakagami[1], Tatsuya Kushida[2], Toru Makino[3],
Tsutomu Hatano[4], Yoshiaki Shirataki[5], Tomohiko Matsuta[1],
Yukiko Matsuo[6] and Yoshihiro Mimaki[6]
[1]Meikai University School of Dentistry, Saitama,
[2]Non-Profit Organization Bio-Knowledge Bank, Tokyo,
[3]HumaLabo Co., Ltd., Tokyo,
[4]Graduate School of Medicine,
Dentistry and Pharmaceutical Sciences, Okayama University, Okayama,
[5]Faculty of Pharmaceutical Sciences, Josai University, Saitama,
[6]Tokyo University of Pharmacy and Life Sciences, School of Pharmacy, Tokyo,
Japan

1. Introduction

Hot water extracts of plants have been utilized to treat various diseases without severe side effects. The presence of numerous kinds of components in the extract made it difficult to identify the causative substances. The hot water extracts contain relatively higher amounts of high-molecular weight polysaccharides and lignin-carbohydrate complexes (LCCs) and relatively lower amounts of low-molecular weight tannins, flavonoids, terpenes and saponins.

Polysaccharides and LCCs are easily extractable by hot-water or alkaline solution and thus be expected to be present in higher amounts in the extract, but the complete determinations of the chemical structures have never been achieved due to the structural complexity. On the other hand, tannins, flavonoids, terpenes and some saponins are mostly difficult to be dissolved in water, but more easily extractable with methanol, and the structures of thousands of compounds have been identified. These low molecular weight compounds are thus expected to be present in lower amounts in the extract. Therefore, the information of how much these lower molecular materials are present in hot-water extract is limited, albeit important.

Based on these circumstances, we analyze the functionality of polysaccharides and LCCs, tannins, flavonoids and saponins as alternative medicines, citing the literatures of other groups and ours, focusing on the following points: (1) yield and putative amounts in methanol and hot-water extracts, (2) biological activity, (3) state in hot-water extract: possible binding to other compounds, (4) site of action, signalling pathway, and receptor identification, and (5) future directions.

2. Lignin-carbohydrate complex (LCC)

Lignins are major class of natural products present in the natural kingdom, and are formed through phenolic oxidative coupling processes in the plant (Davin et al., 1997). Lignins are formed by the dehydrogenative polymerization of three monolignols: *p*-coumaryl, *p*-coniferyl and sinapyl alcohols (Lewis & Yamamoto, 1990). These monolignols were produced from L-phenylalanine by general phenylpropanoid pathway (Emiliani et al., 2009). Some polysaccharides in the cell walls of lignified plants are linked to lignin to form lignin-carbohydrate complexes (LCCs) (Fig.1). Complete structural determination of LCC has not been achieved yet, due to structural complexity. Polysaccharide portions are composed of various types of sugars. For example, LCCs from pine cone of *Pinus parviflora* Sieb et Zucc. contained 11-40% neutral sugars (galactose > glucose > mannose > arabinose or fucose) and 2-58% uronic acid (Sakagami et al., 1987). Similarly, *Lentinus edodes* mycelia extract (LEM) showed similar sugar composition 32% of neutral sugars (glucose > mannose > galactose > arabinose) and 68% uronic acids. Varying the ratio of polysaccharide to phenylpropenoids produces heterogeneity in the acidity, water-solubility, ethanol-insolubility, and molecular weight. LCCs from bald cypress, birch and rice straw show an extremely broad molecular weight distribution from 1.5 to 85 kDa (Azuma & Koshijima, 1988). In their role as integral cell wall components, lignins help provide mechanical support and defend against pathogens. Although the use of dietary fiber (composed of non-α-glucan polysaccharides and lignin) in the treatment of constipation and uncomplicated diverticular diseases is well established (Kay, 1982), very little attention has been paid to the biological activities of lignins or lignin-containing/derived substances.
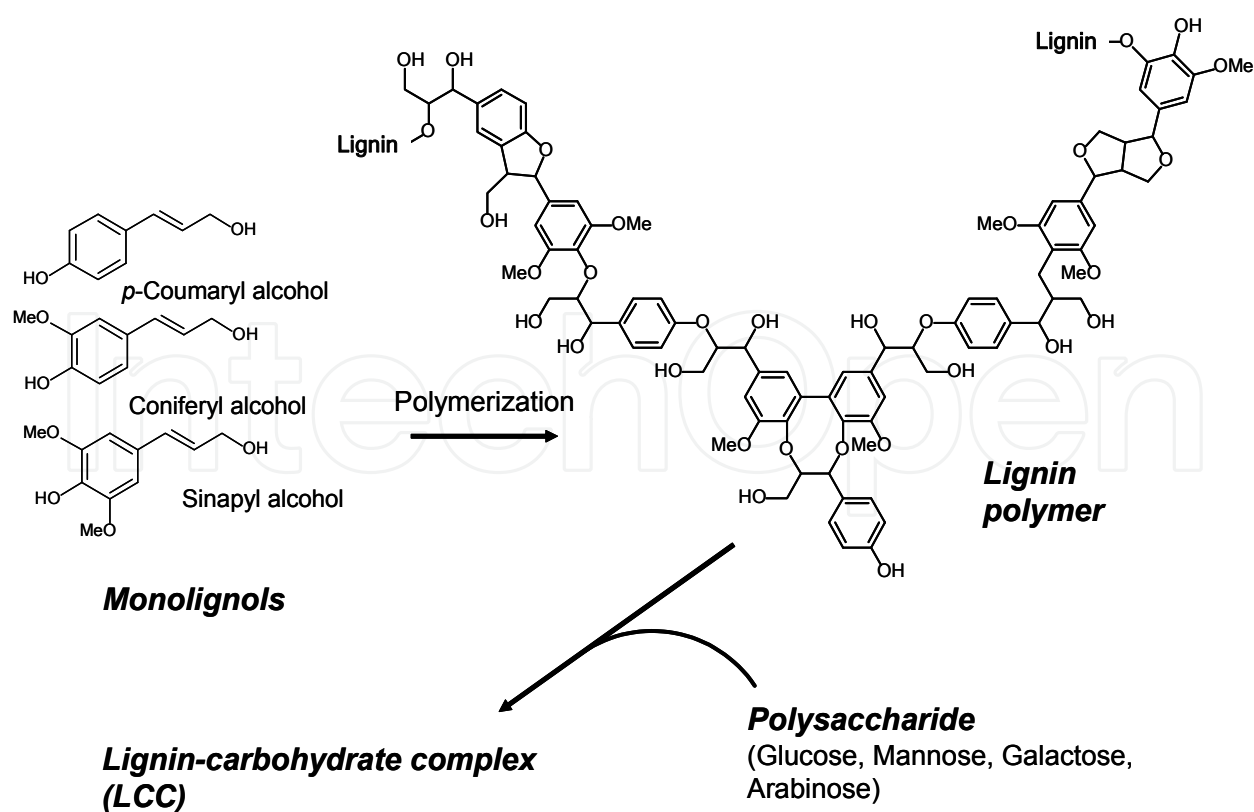

Fig. 1. Pathway of LCC formation.

2.1 Yield and putative amounts in methanol and hot water extracts

Usually, LCCs are extracted by alkaline extract, and concentrated by acid-precipitation. The yield of LCCs are 0.04 to 7.9% of the plant materials (Sakagami et al., 2010a) (Table 2). Gel filtration chromatography of the alkaline extract of *Sasa senanencis* Rehder revealed that LCC represents major parts of the extract (Matsuta et al, 2011). The major part of polyphenols in LCCs from LEM is composed of lignin precursors such as *p*-coumaric acid, vanillic acid, syringic acid and ferulic acid, but not tannin nor flavonoids (Table 1).

	µg/g LEM	µg/g Fr4		µg/g LEM	µg/g Fr4
Lignin precursors			*Tannins and related compounds*		
Vanillic acid	785.5	25.9	Gallic acid	<0.1	<0.1
Syringic acid	638.0	25.7	Catechin	<0.1	<0.1
p-Coumaric acid	277.0	157.7	Epicatechin	<0.1	<0.1
Ferulic acid	74.5	13.4	Epigallocatechin gallate	<0.1	<0.1
Vanillin	59.5	<0.1	Gallocatechin gallate	<0.1	<0.1
Caffeic acid	12.0	<0.1	Epicatechin gallate	<0.1	<0.1
Syringaldehyde	<0.1	<0.1	Catechin gallate	<0.1	<0.1
o-Coumaric acid	<0.1	<0.1	Ellagic acid	<0.1	<0.1
			Theaflavin-3-gallate	<0.1	<0.1
Flavonoids			Theaflavin-3'-gallate	<0.1	<0.1
Fisetin	<0.1	<0.1	Theaflavin-3,3'-gallate	<0.1	<0.1
Daizein	<0.1	<0.1			
Genistein	<0.1	<0.1	*Others*		
Kaempferol	<0.1	<0.1	Eugenol	<0.1	<0.1
Apigenin	<0.1	<0.1	Chlorogenic acid	<0.1	<0.1
Galangin	<0.1	<0.1	Salicylic acid	<0.1	<0.1
Chrysin	<0.1	<0.1	*p*-Hydroxy benzoic acid	737.0	<0.1
Hesperetin	<0.1	<0.1	*o*-Aminobenzoic acid	<0.1	<0.1
Myricetin	<0.1	<0.1	7-Hydroxycoumarin	<0.1	<0.1
LPS		0.0395			

Table 1. Compostion of LCC (Fr4) prepared from *Lentinus edodes* mycelia extract (LEM)

2.2 Biological activity

2.2.1 Immunopotentiating actiivty

Pretreatment of mice with LCC derived from pine cone extract increased the survival time of tumor-bearing mice (Sakagami et al., 1987) and bacterial-inoculated mice, possibly due to the accumulation of polymorphonuclear cells and monocytes/macrophages that produce active oxygens (Harada et al., 1988).

Pretreatment of mice with LCC induced antimicrobial activity against various microorganisms (*Staphylococcus aureus* SH10, *Escherichia coli* GN2411, *Pseudomonas aeruginosa* H7, *Klebsiella pneumoniae* ST101, *Candida albicans* YA2), but not *Salmonella enteritidis* 116-54 (Harada et al., 1988; Oh-hara et al., 1990). When the sugar moiety of LCC was destroyed by

treatment with sulfuric acid or trifluoroacetic acid, the antimicrobial activity was significantly reduced. Furthermore, dehydrogenation polymers of phenylpropenoids, which do not contain sugar, showed much lower antimicrobial activity. These data suggest the importance of sugar moiety of LCC for the induction of antimicrobial activity.

Pretreatment with LCCs protected infant mice from *Hymenoleptis nana* (Cestoda) infection. Subcutaneous administration of LCCs (10 mg/kg) to 1 week old mice evoked strong protective immunity against oral infection by *Hymenolepis nana* eggs. Significant antiparasite effects were also induced in 4 week old mice by intraperitoneal or oral administration of LCCs. Injection of LCCs by any root (*s.c*, *i.p.*, *p.o.*) induced higher antiparasite activity than other natural antitumor polysaccharides [pine cone polysaccharide (Frs. I, II), PSK (protein-bound polysaccharide), Schizophyllan (water-soluble glucan), TAK (water-insoluble glucan), carboxymethyl TAK] (Abe et al., 1989). These data suggest that the conjugation of sugar and polyphenols is essential for the expression of *in vivo* biological activity.

2.2.2 Cytokine production

a. *In vivo* study.

Endogenously produced TNF have been reported to induce resistance against microbial infection (Nakane et al., 1988). When mice were intravenously treated with eliciting agents such as OK-432 (Picibanil)(Sakagami et al., 1990a) or *Lactobacillus casei* (Sakagami et al., 1992a), TNF was induced in the blood, reaching a maximum level after 2-3 hours, and thereafter rapidly declined. Priming the mice with LCC, much higher level of endogenous TNF was induced, accompanied by hepatic accumulation of Kupffer cells. The priming effect of LCC may be involved in the induction of antitumor, antimicrobial and antiparasite activities. The endogenous TNF production primed by LCC decreased during the aging of mice and upon the tumor implantation into the mice (Hanaoka et al., 1989), suggesting the possibility that the resistance of the hosts against microbial infection may decline with aging.

b. *In vitro* study.

In contract, contamination of lipopolysaccharide (LPS) at more than 0.0001% of sample significantly affected cytokine determination *in vitro*. When the biological activity derived from contaminating LPS was subtracted, both cacao mass and husk LCCs lost the stimulation activity of nitric oxide (NO) and cytokine (TNF-α, IL-1β, IFN-γ) production in mouse macrophage-like cell lines (RAW264.7, J774.1). However, cacao mass LCC enhanced LPS-induced iNOS expression in RAW264.7 cells. These data demonstrated several new biological activities of LCC distinct from LPS and further confirmed the promising antiviral and immunomodulating activities of LCCs (Sakagami et al., 2011).

2.2.3 Anti-viral activity

a. Anti-HIV activity:

Anti- human immunodeficiency virus (HIV) activity was assessed quantitatively by a selectivity index ($SI=CC_{50}/EC_{50}$, where CC_{50} is the 50% cytotoxic concentration against mock-infected MT-4 cells, and EC_{50} is the 50% effective concentration against HIV-infected cells).

LCCs from pine cone from *Pinus parviflora* Sieb et Zucc. and *Pinus elliottii* var. Elliotti (SI=14, 28), bark of *Erythroxylum catuaba* Arr. Cam. (SI=43) (Manabe et al., 1992), husk and mass of *Theobroma cacao* (SI= 311, 46) (Sakagami et al., 2008) and cultured extract of LEM (SI=>94) (Kawano et al. 2010) and mulberry juice (SI=7) (Sakagami et al., 2007; Sakagami and Watanabe, 2011) showed potent anti-HIV activity. The anti-HIV activity of the hot water extract of LEM has been demonstrated also by other groups (Suzuki et al., 1990). The anti-HIV activity of LCCs (Nakashima et al., 1992a) was generally higher than that of lower molecular weight polyphenols, such as tannins (SI=1-11) (Nakashima et al., 1992b), flavonoids (SI=1) (Fukai et al., 2000), gallic acid, (-)-epigallocatechin 3-*O*-gallate (EGCG), curcumin, and natural and chemically modified glucans [*N,N*-dimethylaminoethyl paramylon, *N,N*-diethylaminoethyl paramylon, 2-hydroxy-3-trimethylammoniopropyl paramylon, sodium caroboxymethyl paramylon, carboxymethyl-TAK) (SI=1) except for sulfated polysaccharide (such as paramylon sulfate and dextran sulfate) (Koizumi et al., 1993) (Table 2).

	Yield (%)	Anti-HIV activity (SI)
LCC pine cone of *Pinus parviflora* Sieb. et Zucc.	0.51	14
LCC from pine cone of *Pinus elliottii* var. Elliottii	0.35	28
LCC from seed shell of *Pinus parviflora Sieb. et Zucc.*	0.1	12
LCC from bark of *Erythroxylum catuaba* Arr. Cam.	0.19	43
LCC from husk of *Theobroma cacao*	3.2	311
LCC from mass of *Theobroma cacao*	7.9	46
LCC from cultured *Lentinus edodes* mycelia	0.54	94
LCC from mulberry juice	1.9	7
Dehydrogenation polymers of phenylpropenoids (n=23)		105
Neutral polysaccharide from pine cone	0.1	1
Acidic polysaccharide from pine cone	0.81	1
N,N-dimethylaminoethyl paramylon (SR: 3.7-6.3%)		<1
N,N-diethylaminoethyl paramylon (SR: 10%)		<1
2-hydroxy-3-trimethylammoniopropyl paramylon (SR: 4.3%)		<1
Paramylon sulfate (SR: 0.08%)		<1
Paramylon sulfate (SR: 4.1%)		>274
N,N-dimethylaminoethyl curdlan (SR: 5.0%)		<1
PSK (protein-bound polysaccharide)		1
Hydrolyzable tannins monomer (n=21) (MW: 484-1255)		<1
Hydrolyzable tannins dimer (n=39) (MW: 1571-2282)		<1
Hydrolyzable tannins trimer (n=4) (MW: 2354-2658)		3
Hydrolyzable tannins tetramer (n=3) (MW: 3138-3745)		11
Condensed tannins (n=8) (MW: 290-1764)		<1
Flavonoids (n=160) (MW: 84-648)		<1
Gallic acid (MW170)		<1
(-)-Epigallocatechin 3-*O*-gallate (MW458)		<1
Curcumin		<1

Table 2. Anti-HIV activity of polyphenols.

Limited digestion of lignin structure by $NaClO_2$ resulted in significant loss of anti-HIV activity (from SI=14 to 3), whereas removal of the monosaccharide residues by acid-catalyzed hydrolysis did not significant affect the anti-HIV activity (from SI= 14 to 13) (Lai et al., 1992), suggesting that phenylpropenoid polymer, but not sugar moiety, is important for anti-HIV activity. Dehydrogenation polymers of phenylpropenoids without carbohydrate showed generally higher anti-HIV activity (SI=105) than LCCs (Nakashima et al., 1992a). On the other hand, phenylpropenoid monomers (*p*-coumaric acid, ferulic acid, caffeic acid) were inactive, suggesting the importance of highly polymerized structure. LCCs inhibited HIV adsorption to the cells (Nakashima et al., 1992a) and the HIV-1 reverse transcriptase activity (Lai et al., 1990) and HIV-1 protease activity (Ichimura et al., 1999).

b.   Anti-influenza virus activity:

LCCs inhibited the plaque formation and RNA polymerase (enzyme engaged in viral replication) activity of influenza virus (Nagata et al., 1990). Limited digestion demonstrated that phenylpropenoid polymer, but not sugar moiety, is important for anti-influenza virus activity (Harada et al., 1991). This was confirmed by our finding that dehydrogenation polymers of phenylpropenoids without carbohydrate inhibited both the plaque formation and RNA polymerase more effectively than LCCs (Sakagami et al., 1990b).

LCC instantly adsorbed to the influenza virus when mixed *in vitro*, as demonstrated by sucrose gradient centrifugation, and abrogated the infectivity of the influenza virus in mouse infection model (Sakagami et al, 1992b).

c.   Anti-HSV activity:

LCCs, isolated from the cones of various pine trees (*Pinus parviflora* Sieb. and Zucc, *Pinus densiflora* Sieb. et Zucc., *Pinus thunbergii* Parl., *Pinus elliottii* var. Elliotti, *Pinus taeda* L., *Pinus caribaea* var. Hondurenses, *Pinus sylvestris* L., and the pine seed shells of *Pinus parviflora* Sieb. et Zucc., and *Pinus armandii* Franch) inhibited the plaque formation of herpes simplex virus types 1 and 2 (HSV-1, HSV-2) strains in African green monkey kidney cells and human adenocarcinoma cells. LCC (pine cone Fr. VI) showed the highest selectivity index (SI=1000) (CC_{50}>300 μg/mL, EC_{50}=0.3 μg/mL) (100% inhibition at 10 μg/mL). On the other hand, neutral polysaccharide (pine cone Fr. I) (0% inhibition at 10 μg/mL) and acidic polysaccharide (rich in uronic acid) (pine cone Fr. II) (0% inhibition), glucans (paramylon, Schizophyllan, TAK), and chemically modified derivatives (*N,N*-dimethylaminoethyl paramylon, sodium carboxymethyl paramylon, sodium paramylon sulfate, carboxymethyl-TAK) and PSK were inactive (Fukuchi et al., 1989a). Tannins such as oenothein B, coriariin A, rugosin D, cornusiin A, tellimagrandin I, casuarictin, penta-*O*-galloyl-β-D-glucose, geraniin, 4,8-tetramer of epicatechin gallate showed potent anti-HSV activity (Fukuchi et al., 1989b), although they showed much weak anti-HIV activity. An experiment using ^{3}H-thymidine labeled virus particles indicated that the anti-HSV effect of both LCCs (Fukuchi et al., 1989a,) and tannins (Fukuchi et al., 1989b) was attributable to interference with virus adsorption to these cells rather than to inhibition of virus penetration into the cells.

Recently, carboxylated lignins, synthesized using 4-hydroxy cinnamic acid scaffold by enzymatic oxidative coupling, have been reported to inhibit the entry of HSV-1 entry into the cells (Thakkar et al., 2010). Sulfated LCC (PPS-2b) (MW8500) also showed anti-HSV activity possibly by inhibiting the viral binding and penetration into host cells. Prunella

cream formulated with a semi-purified fraction significantly reduced the skin lesion and mortality induced by HSV-1 infection in Guinea pigs (Zhang et al., 2007) The anti-HSV activity of sulfated lignins depended on their molecular weight, with the maximum at 39.4 kDa (Raghuraman et al., 2007).

d.   Clinical application.

A clinical pilot study was carried out to evaluate anti-HSV-1 activity of a pine cone LCC and ascorbic acid treatment, with forty eight healthy patients of both genders between 4 and 61 years old (mean: 31 years), with active lesions of HSV-1. According to the HSV-1 stage at the presentation the patients were classified into the prodromic, erythema, papule edema, vesicle/pustule and ulcer stages. One mg of LCC-ascorbic acid tablet or solution was orally administered three times daily for a month. The patients who began the LCC-ascorbic acid treatment within the first 48 hours did not develop HSV-1 characteristic lesions, whereas those patients who began the treatment later experienced a shorter duration of cold sore lesions and a decrease in the symptoms. The majority of the patients reported the reduction in the severity of symptoms and the reduction in the recurrence episodes after the LCC-ascorbic acid treatment compared with previous episodes, suggesting its possible applicability for the prevention and treatment of HSV-1 infection (López et al., 2009).

2.2.4 Synergistic action with vitamin C

Vitamin C exhibited either antioxidant or prooxidant activity, depending on the concentration (Sakagami et al., 2000b). We have reported that ascorbate derivatives that produced the doublet signal of ascorbate radical (sodium-L-ascorbate, L-ascorbic acid, D-isoascorbic acid, 6-β-D-galactosyl-L-ascorbate, sodium 5,6-benzylidene-L-ascorbate) induced apoptosis (characterized by internucleosomal DNA fragmentation and an increase in the intracellular Ca^{2+} concentration) in HL-60 cells. On the other hand, ascorbate derivatives that did not produce radicals (L-ascorbic acid-2-phosphate magnesium salt, L-ascorbic acid 2-sulfate and dehydroascorbic acid) did not induce apoptosis (Sakagami et al., 1996a, 1996b). This suggests the possible involvement of the ascorbate radical in apoptosis-induction by ascorbic acid-related compounds.

We accidentally found that LCCs from the pine cone of *Pinus parviflola* Sieb et Zucc, pine cone of *Pinus elliottii* var. Elliotti, leaf of *Ceriops decandra* (Griff.) Ding Hou and, thorn apple of *Crataegu Cuneata* Sieb. et Zucc modulated the radical intensity of ascorbate bi-phasically, depending on the concentrations. At higher concentration, LCCs strongly enhanced the radical intensity of sodium ascorbate, which rapidly decayed, possibly due to the breakdown of ascorbic acid or to the consumption of ascorbyl radical. LCCs, not only from pine cones (Fr. VI), but also from Catuaba bark, pine seed shell, *A. nikoense* Maxim. and *C. Cuneata Sieb*. et Zucc. enhanced the radical intensity and cytotoxic activity of sodium ascorbate (Sakagami et al., 2005). On the other hand, tannins such as gallic acid, EGCG, and tannic acid counteracted the radical intensity and cytotoxic activity of sodium ascorbate (Satoh et al, 1999).

Sodium ascorbate rapidly reduced the oxygen concentration in the culture medium, possibly due to oxygen consumption *via* its pro-oxidation action. Simultaneous addition of LCCs further enhanced the ascorbate-stimulated consumption of oxygen (Sakagami et al., 1997). These data suggest that the synergistic enhancement of the cytotoxic activity of LCCs and ascorbate might be due at least in part to the stimulated induction of hypoxia.

Lower concentration of LCC (pine cone Fr. VI) and sodium acorbate showed radical scavenging activity. LCC further stimulated the superoxide anion (O_2^-) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of sodium ascorbate. LCCs from *Ceriops decandra* (Griff.) Ding Hou. and cacao husk scavenged O_2^- and hydroxyl radical, and synergistically enhanced the radical scavenging activity of sodium ascorbate (Sakagami et al., 2005, 2008) .

2.3 State in hot-water extract: Possible binding to other compounds

Solvent fractionation of Alkaline Extract of the Leaves of *Sasa senanensis* Rehder (SE) demonstrated that (i) chlorophyllin in SE was recovered from the water layer, that contains majority of compounds (more than 81%) and inhibited the NO production by macrophages more potently than other *n*-hexane, diethyl ether and ethylacetate layers (Sakagami et al., 2010). Three-dimensional HPLC analysis demonstrated that the majority of SE components are recovered from one major peak. Furthermore, LCC isolated from SE showed the unique greenish color of chlorophyllin (absorption maximum = 452 nm) (Sakagami et al., 2010a). These data strongly suggest the possible association of chlorophyllin with LCC in the native state or during extraction with alkaline solutions.

2.4 Site of action, signaling pathway, and receptor identification

A receptor for LCC, and the related signal transduction pathways had been largely unknown. On the other hand, β-glucans and α-mannans, which constitute the cell wall of fungi and plants, are recognized by dectin-1 and dectin-2, respectively, which are members of C-type lectins, and prominently expressed in the cell membrane of dendritic cells and macrophages as the transmembrane receptors (Brown & Gordon, 2001; McGreal et al., 2006; Vautier et al., 2010). It is also known that dectin-1 and 2 are essential for host defense against fungi, such as, *Pneumocystis carinii*, and *Candia albicans* (Saijo et al., 2007; 2010). Furthermore, genetic and immunological studies have demonstrated the molecular mechanism regarding the immunostimulation and phylaxis recently. It became evident that, in the dendritic cells and macrophages, activated dectin-1 and 2 by β-glucans and α-mannans, respectively, can induce the activation of NFκb thorough signal transduction molecules such as syk and Card9-Bcl10-Malt1 (Gross et al., 2006; Saijo et al., 2010; Vautier et al., 2010).

In order to elucidate their action point, DNA microarray analysis was performed, using mouse macrophage-like J774.1 cells. RNA was isolated with Qiagen RNeasy Plus Mini kit, hydrized with GeneChip MouseGene 1.0 ST arrays, and scanned with Affymetrix GeneChip Command Console software. One of the seven LCC fractions isolated from LEM (Fr4) enhanced the expression of dectin-2 (4.2-fold) and toll-like receptor (TLR)-2 (2.5-fold) prominently, but only slightly modified the expression of dectin-1 (0.8-fold), complement receptor 3 (0.9-fold), TLR1, 3, 4, 9 and 13 (0.8- to 1.7-fold), spleen tyrosine kinase (Syk)b, zeta-chain (TCR) associated protein kinase 70kDa (Zap70), Janus tyrosine kinase (Jak)2 (1.0- to 1.2-fold), nuclear factor (Nf)κb1, NFκb2, reticuloendotheliosis viral oncogene homolog (Rel)a, Relb (1.0- to 1.6-fold), Nfκbia, Nfκbib, Nfκbie, Nfκbi12 Nfκbiz (0.8- to 2.3-fold). On the other hand, LPS did not affect the expression of dectin-2 nor TLR-2 (Fig. 2). These data suggest the significant role of the activation of the dectin-2 signaling pathway in the action of LCC on macrophages (Kushida et al., 2011).
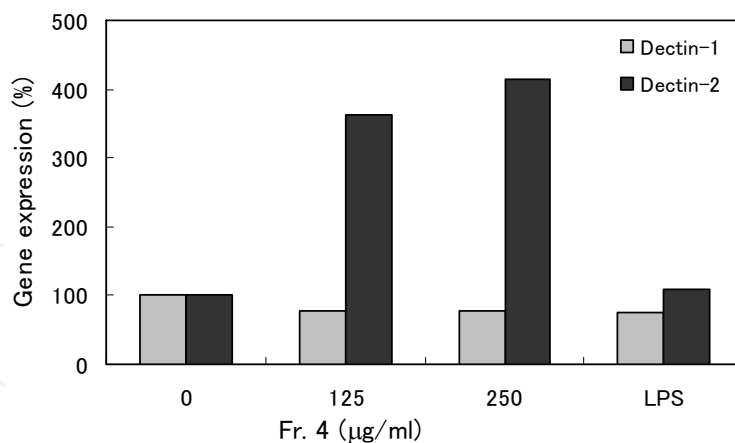
Fig. 2. Enhancement of *dectin-2* gene expression by LCC from LEM. Modified from Kushida et al., 2011, with permission.

2.5 Future directions

We have previously reported that tumor-specificity of oligomeric tannins (both hydrolysable and condensed types) is higher than that of monomeric tannins (Sakagami et al., 2000a), although the tumor-specificity of tannins was much lower than that of chemotherapeutic drugs (Sakagami, 2010a). This suggests the importance of determining the optimal molecular weight of LCC for the expression of the highest biological activity. However, with the increase in the molecular weight, the water-solubility of LCC may decline, and make it difficult to be sterilized by millipore filteration.

There is a possibility that bacterial endotoxin (LPS) in the soil and air may contaminate LCC during the isolation step, since LPS is similarly extracted with alkaline solution and precipitated with acid. Most of previous studies have not paid attention to such LPS contamination in the LCC preparations. Alkaline extraction step that is necessary for the preparation of LCC has both merit and demerit. Merit is the chemical inactivation of LPS. Demerit is the degradation of LCC into its smaller size. Therefore, the conditions for alkaline extraction should be optimized to maximize the LPS inactivation and minimize the loss of biological activity.

It is generally accepted that lignin is linked by various amounts of polysaccharides (polymers of glucose or other sugar), yielding LCCs having broad ranges of molecular weights. Phenylpropenoid polymer portion of LCC have potent anti-vial activity, whereas carbohydrate portion have immunopotentiation activity, possibly *via* activation of TLR pathway and chemokine expression (Fig. 3). After extraction with alkaline solution, the structure of LCC may be either unmodified or modified. When LCC is prepared from the leaves, chlorophyllin may be associated during extraction step with alkaline solution. This association may enhance anti-inflammation activity and inhibition of cytochrome P-450 (CYP). When washing procedure is incomplete, LPS from bacteria in the soil and air may associate with LCC, considering that both LPS and LCC are precipitated by acid. This association may trigger the back-ground level of immune response-related genes expression. The associations with chlorophyllin, LPS and others may produce diverse biological activities, depending on the plant species and preparative procedures.
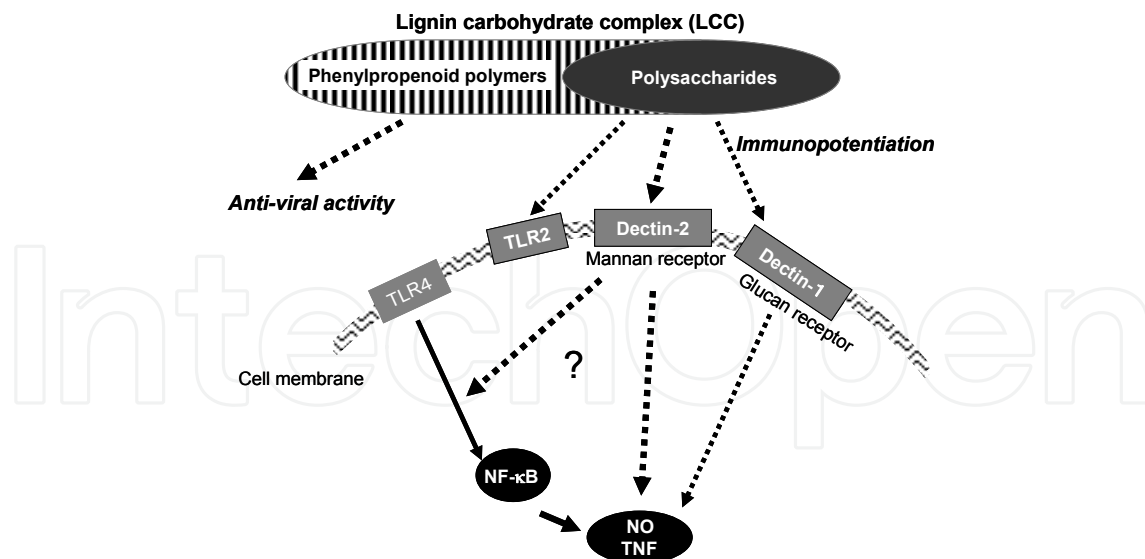
Fig. 3. Possible site of action of LCC

3. Tannins

3.1 Yield and putative amounts in methanol and hot water extracts

Tannins are defined to be naturally occurring polyphenols with potent binding activity with proteins and other biomolecules such as polysaccharides and lipids. The binding of tannins with those molecules is attributed to the hydrogen bonding and/or hydrophobic interaction, in addition to electrostatic interaction. In some cases covalent bonding also attributes to the interaction with proteins. Tannins are structurally classified into two major groups. Those belonging to one group are called hydrolysable tannins: i.e., esters of gallic acid and its oxidative derivatives with glucose or related sugars. The other ones are belonging to condensed tannins: i.e., flavan oligomers or polymers where their constituent monomeric flavans are connected mainly by C-4 – C-8 or C-4 – C-6 linkages. Some other types of polyphenolic compounds such as those containing several caffeic acid units (caffeetannins), and phloroglucinol oligomers or polymers (phlorotannins) are also regarded to be tannins. The binding properties of tannins, and therefore their biological and/or pharmacological properties attributed to the binding, vary depending on their structures. Since tannins bind strongly with cellulose and lignin of plant cells with hydrogen bonding and hydrophobic interaction, combinations of acetone and water are used as the solvents for extraction of tannins to release them from the plant cells. Some examples for the extractability of tannins from plant materials are shown below.

Geranium thunbergii is a plant species belonging to the family Geraniaceae, and its aerial part has been used as a folkmedicine for treatments of intestinal disorders including diarrhoea in Japan. Geraniin, the major constituent of this medicinal herb, is the representative hydrolysable tannin composed of galloyl, hexahydroxydiphenoyl (HHDP), and dehydrohexahydroxydiphenoyl (DHHDP) groups, in addition to a glucopyranose core. Several other tannins with related structures, including didehydrogeraniin, furosin, furosinin, elaeocarpusin (=ascorgeraniin), and geraniinic acids B and C, were also isolated from the same source plant species. Quantitative analysis of this medicinal herb using methylene blue for the estimation of total tannin indicated that the tannin contents vary 0.9

% - 2.4 % of the aerial part, depending on the collected month. The quantitative experiments applied to several *Geranium* species indicated that the extractability for tannins with methanol is lower than that with the mixture of acetone and water (ca. 30-50% lower). The quantitative analysis of geraniin, the major tannin of *G. thunbergii*, by high-performance liquid chromatography (HPLC) of the aqueous acetone extracts indicated that the contents were 0.6 % - 1.8 % of the aerial parts of the plant species (Okuda et al., 1980).

Most of the medicinal herbs have been extracted with hot water used in the traditional medicine, although some types of constituents are decomposed in hot water to give various products. Geraniin is also easily hydrolyzed in water to give several lower molecular weight polyphenols, such as corilagin, gallic acid, ellagic acid, and brevifolin (Fig. 4). Thus, the behaviour of geraniin during the extraction of the constituents from this medicinal herb with hot water was investigated. The first increment of the geraniin concentration was then followed by its decrease accompanied by the increase of corilagin, a hydrolysis product from geraniin. The highest concentration of geraniin during the decoction was observed at the time just after the boiling.
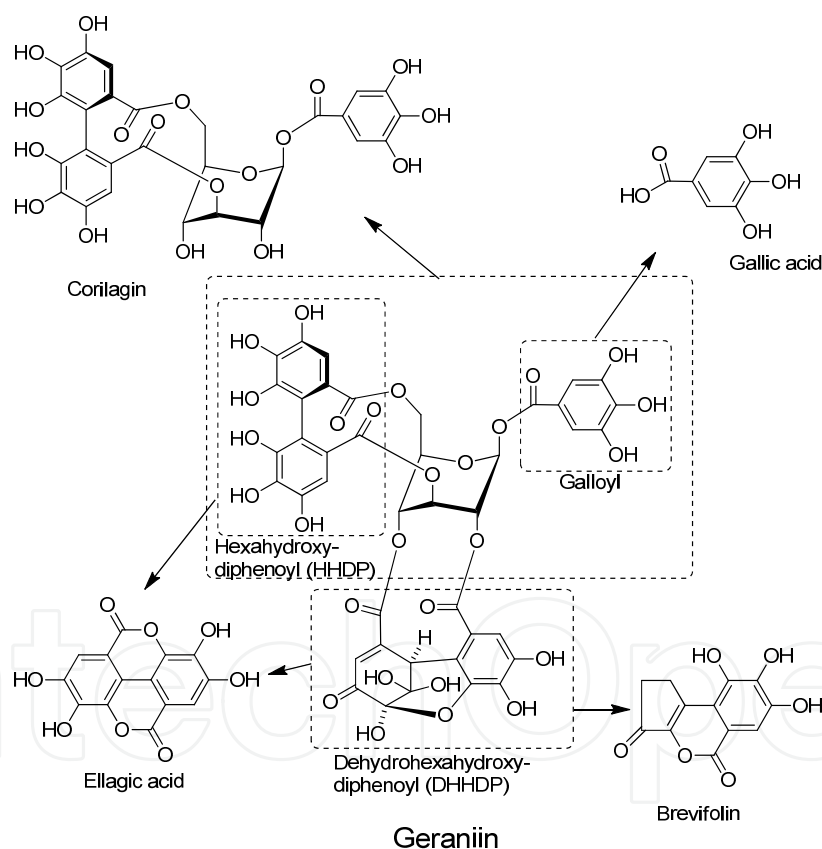

Fig. 4. Structural changes of geraniin in hot water

Analogous quantitative analysis was also performed for the extraction of rosmarinic acid (Fig. 5), called Labiatae-tannin, from dried leaves of *Perilla frutescens*. As a result, the concentration of this polyphenolic compound increased until the time of the boiling. The concentration did not decrease during the continuous heating of the aqueous solution, while rosmarinic acid was quite unstable when preparing the dried herb by heated air (Okuda et al., 1986).
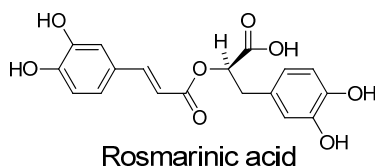
Fig. 5. Structure of rosmarinic acid

3.2 Biological activity

3.2.1 Anti-tumor activity

Some hydrolysable tannins with high molecular weights, including oenothein B, have been shown to have potent host-mediated anti-tumor activity. Recently, we found that several hydrolysable tannins isolated from plants belonging to the family Tamaricaceae, such as nilotinin D8 (Fig. 6), showed remarkable cytotoxic effects on cultured cell lines from oral tumors (Orabi et al. 2010).
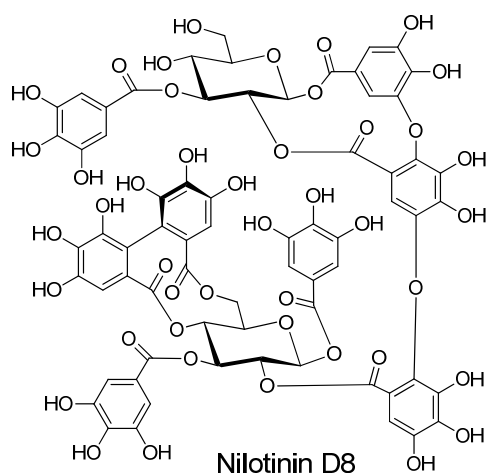

Fig. 6. Structure of a *Tamarix* tannin effective on the tumor cells

3.2.2 Anti-bacterial activity

Antibacterial effects of polyphenolic compounds including tannins have been revealed for various bacterial species. One of the important problems concerning the infectious disease is the menace of antibiotic resistance. Clinical usages of antibiotics against infectious diseases have caused developments of antibiotic resistance of the bacteria. Among various antibiotic-resistant bacteria species, methicillin-resistant *Staphylococcus aureus* (MRSA) causes about 20 000 or more patients in a year in Japan. Tannins showed potent effects on the antibiotic resistance of bacteria as shown below.

Clinical isolates of MRSA found in Okayama University Hospital, which acquired antibiotic resistance against β-lactams, aminoglycosides, macrolides, and some other antibiotics, were used as target bacteria. The addition of (-)-epicatechin gallate (25 mg/L), isolated from green tea leaves, decreased the minimum inhibitory concentration (MIC) of oxacillin, 128-512 mg/L for four MRSA strains, to 0.5-1 mg/L (Shiota et al., 1999). An analogous decrease of MIC of oxacillin was found for the addition of tellimagrandin I, a hydrolysable tannin isolated from rose petals. The addition of tellimagrandin I also brought the decrease of the

MIC of tetracycline for two MRSA strains, from 2 to 0.25, and 8 to 1 mg/L, respectively (Shiota et al., 2000). Corilagin, isolated from leaves of *Arctostaphyllos uva-ursi*, also caused noticeable decreases of the MIC values of several β-lactam antibiotics. The mechanisms of the effects of the hydrolysable tannins were also examined, and the suppression of the function of penicillin-binding protein 2a (PBP-2a), and also the inhibitory effects on β-lactamase have been suggested (Shimizu et al., 2001).

Further investigation revealed that a product obtained by incubation of (-)-epigallocatechin gallate (EGCG) in solution showed lowering effects on MIC of β-lactam antibiotics. Since EGCG is unstable even in neutral solution, we investigated the changes in the structure of EGCG, and found formation of dimeric products. Among the products, theasinensin A (Fig. 7) caused marked decrease of MIC of oxacillin, and related β-lactam antibiotics. MIC of streptomycin for the MRSA strains and for a methicillin-sensitive *Staphylococcus* strain decreased upon the addition of theasinensin A (Hatano et al., 2003a). Condensed proanthocyanidin obtained from pericarpus of Japanese pepper (*Zanthoxylum piperitum*) also decreased MIC of β-lactam antibiotics (Kusuda et al., 2006).
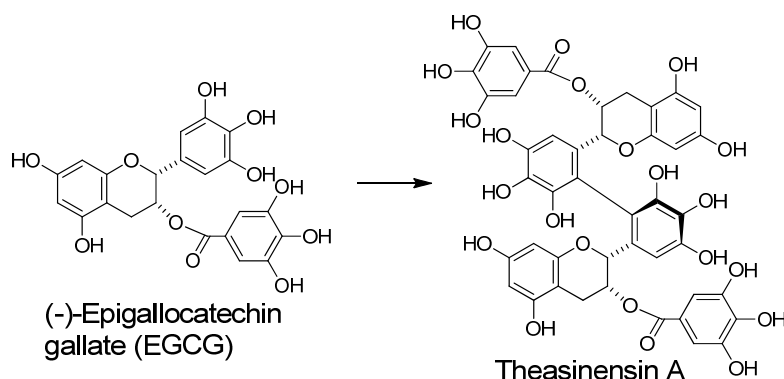

(-)-Epigallocatechin gallate (EGCG)

Theasinensin A

Fig. 7. Structural changes of EGCG into theasinensin A

3.3 State in hot-water extract: Possible binding to other compounds

Although some hydrolysable tannins such as geraniin were unstable in water as mentioned above, the stability of hydrolysable tannins in water is dependent on their structures. Structural changes of different oligomeric hydrolysable tannins during the hot water treatment were investigated, and differences in the instability of those tannins have also been shown. For example, isorugosin D, which is a regio-isomer of rugosin D concerning the orientation of valoneoyl group, was more stable, relative to rugosin D, against the hot-water treatment. Oenothein B, which has a macrocyclic structure with two valoneoyl groups in the molecule, was much more stable relative to those dimers (Fig. 8) (Yoshida et al., 1992).

Investigation on the metabolic profiles after oral administration of galloylglucoses revealed formation of 4-*O*-methylgallic acid. The HHDP group in ellagitannins is metabolised into urolithins A and B, and some compounds structurally related to them (Ito et al., 2008). On the other hand, metabolism of catechins and related oligomeric flavanoids gives protocatechuic acid, and its methyl derivatives (Fig. 9). Our investigation of the binding of EGCG and those low-molecular-weight metabolites revealed that they affects the bindings of site-I- and site-II-binding drugs to human serum albumin, depending on their structures (Nozaki et al., 2009).
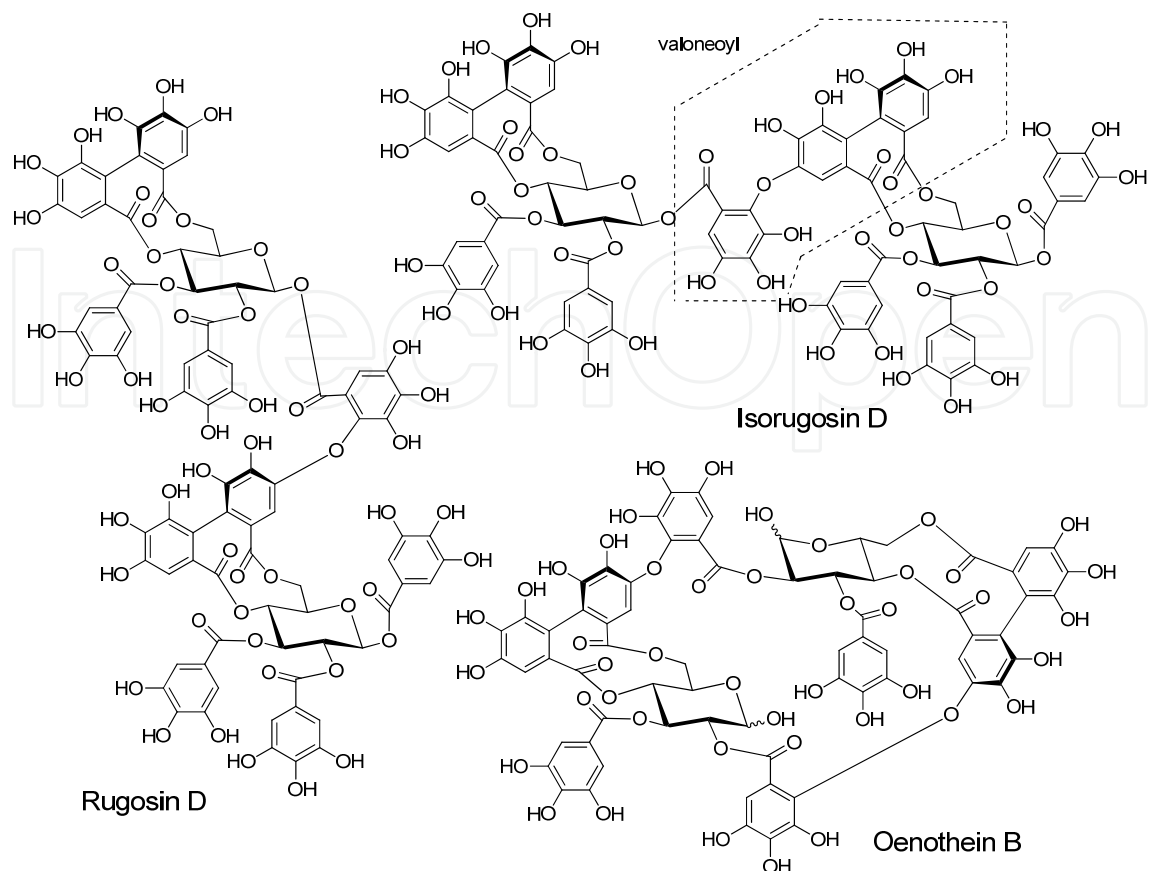
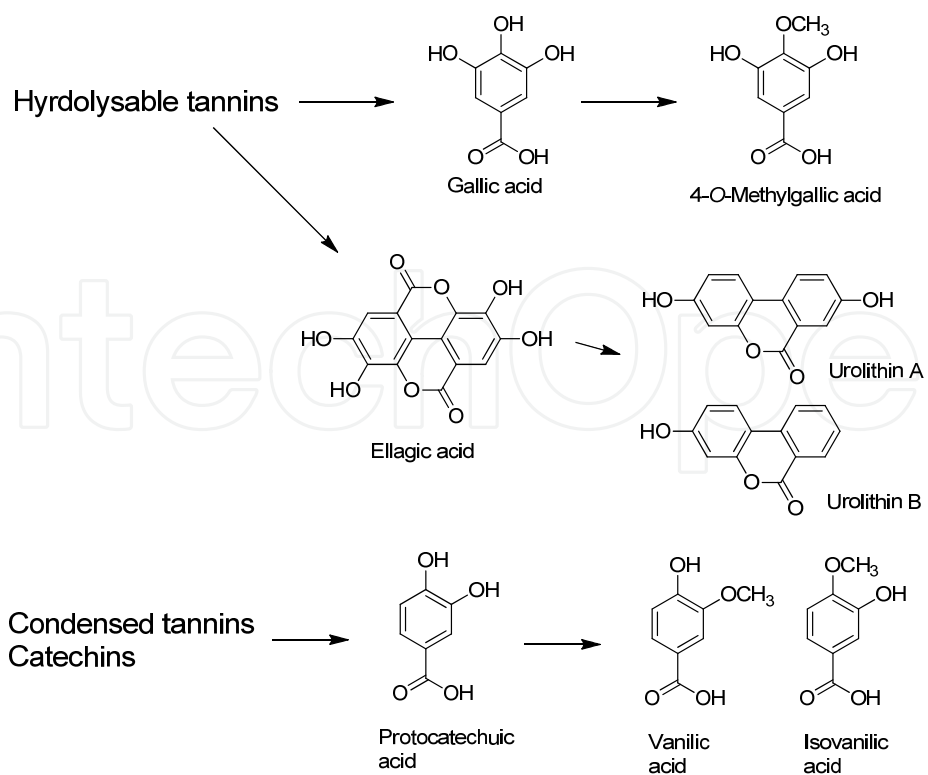Fig. 8. Structures of rugosin D, isorugosin D, and oenothein B

Fig. 9. Metabolism of tannins

3.4 Site of action, signaling pathway, and receptor identification

Pentagalloyglucose has been reported to induce autophagic cell death in prostate cancer cells (Hu et al., 2009) and inhibit its bone metastasis by transcriptionally repressing EGF-induced metalloproteinase secrtion (Kuo et al., 2009). On the other hand, proanthocyanidines induce apoptotic cell death in human non-small cell lung cancer cells, at least in part due to inhibition of COX-2, PGE_2 and PGE_2 receptor expression (Shama et al., 2010).

3.5 Future directions

Many researchers have reported various pharmacological and biological activity of polyphenolic compounds including tannins. However, some of those compounds, especially several tannins and condensed proanthocyanidins or catechins are unstable in water and/or on heating. Therefore, the actual pharmacologically active compounds may be different from the administered compounds. The changing procedures should be clarified. Our study on the structural changes of catechins will give a solution of the problem (Taniguchi et al., 2008).

Binding of polyphenolic compounds, especially the binding of tannins, to biomolecules should be further investigated, too, since their pharmacological activity starts with the association or complex formation of the polyphenols and biomolecules. Our study on the formation of complex from tannins and related polyphenols with serum albumin revealed that the formed supermolecule has the "molecular weight" of 9.5×10^5 for a combination (Hatano et al., 2003b). The structural details of those supermolecules are still in an enigma.

4. Flavonoids

Flavonoids are important secondary metabolites in higher plants, and classified into flavones, isoflavones, flavonols, flavanones, catechins and anthocyanidins, based on the skeletons of aglycone moieties (Fig. 10). Backbone structures of all flavonoids are derived from two metabolites, malonyl-CoA and *p*-coumaroyl-CoA, and the biosynthetic pathway of flavonoids are the condensation of three molecules of malonyl-CoA with one molecule of *p*-coumaroyl-CoA (Martens & Mithöfer, 2005).

Flavone glycosides are classified into *O*- and *C*-glycoside, and flavone *C*-glycosides are found in several plants such as *sasa* genus (Nakajima et al., 2003, Park et al., 2007), *cucurbitaceae* (Abou-Zaid et al., 2001) and buckwheat seedling (Watanabe and Ito, 2002). Flavonols such as kaempferol and quercetin glycosides, flavones such as apigenin and luteolin glycosides and anthocyanidins (Fig. 10) display several biological activities.

4.1 Yield and putative amounts in methanol and hot water extracts

Flavonoids of yellow pigment are usually extracted by methanol, ethanol or alcohol-water mixtures, and fractionated by diethyl ether and ethyl acetate. Anthocyanines of red pigment are usually extracted by methanol and hydrochloric acid or trifluoroacetic acid mixtures (with yield of about 1-5%). The use of mineral acid can lead to the loss of attached acyl group.
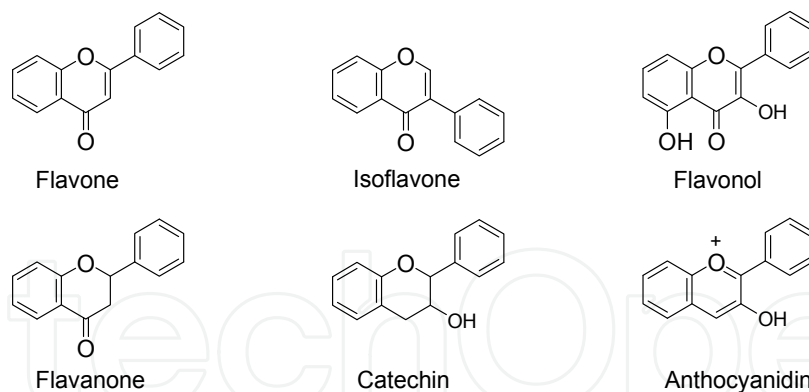
Fig. 10. Basic structures of flavonoids.

4.2 Biological activity

4.2.1 Anti-osteoporosis activity

Osteoporosis is considered to be one of the hormonal deficiency diseases observed in the menopausal women and elderly persons. When estrogen is reduced in the body, the production of pro-inflammatory cytokines such as IL-1β and IL-6 are elevated, and the osteoclastogenesis enhanced, leading to the bone resorption. Glucosidic isoflavones of PIII (Isocal, glucosidic isoflavones extracted from *Sophorae fructus*) stimulated the osteoblastic proliferation by suppressing the IL-1β and IL-6 production and elevating the NO level. In bone marrow primary culture, PIII effectively suppressed the osteoclastogenesis. These effects of PIII was slightly lower than 17β-estradiol, but higher than two soybean isoflavones [daidzin (**1**), genistin (**2**)]. PIII may preferentially induce anti-osteoporosis response by attenuating their osteoclastic differentiation and by upregulating the NO (Fig.11) (Joo et al., 2003).
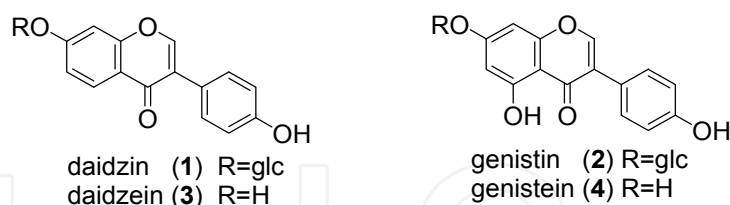

daidzin   (**1**) R=glc
daidzein (**3**) R=H

genistin   (**2**) R=glc
genistein (**4**) R=H

Fig. 11. Two glucosidic isoflavones of daidzin (1) and genistin (2)

4.2.2 Tumor-specificity evaluated in vitro

Prenylflavanones [sophoraflavanone G (**5**), sophoraflavanone B, sophoraflavanone A, euchrestaflavanone A, sophoraflavanone H (**6**), sophoraflavanone I (**7**)](Fig. 12) showed higher cytotoxicity agaist two human oral tumor cell lines (HSC-2, HSG), as compared with normal gingival fibroblast (HGF) . Prenylflavanones having either prenyl-, lavandulyl- or geranyl groups on A-ring, and two flavonostilbenes having stilbene (resveratrol) on B-ring showed tumor-specific cytotoxicity, radical generation and O₂⁻ scavenging activity.

Isoflavones with two isoprenyl groups (one in A-ring and the other in B-ring) such as tetrapterol G and isosophoranone, and isoflavanones with α,α-dimethylallyl group at C-5' of B-ring such as secundifloran, secundiflorol A, secundiflorol D and secundiflorol E had their

relatively higher cytotoxic activity. Secundiflorol A had the highest tumor-specific cytotoxicity (tumor specicicity index (TS)=2.8), followed by genistein (TS=2.4) (**4**), secundiflorol D (TS=1.9), secundiflorol E (TS=1.9), secundiflorol F (TS=1.9) > sophoraisoflavanone A (TS=1.8), sophoronol (TS=1.7), secundifloran (TS=1.7) > tetrapterol G (TS=1.6) > isosophoranone (TS=1.5) > daidzein (TS=1.1) (**3**). 6,8-Diprenylation of genistein further enhanced the cytotoxicity *via* radical-mediated oxidation mechanism, but reduced the tumor-specificity.Their cytotoxicity became maximum at a log P value of around 4 (Shirataki et al., 2001a, 2001b).
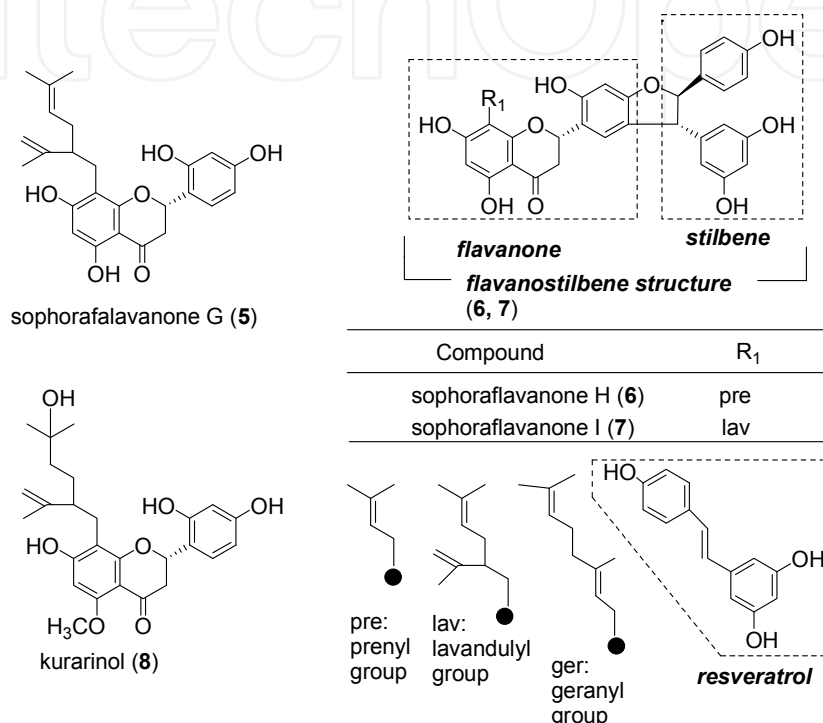

Fig. 12. Prenylflavanones (**5-8**) from *Sophora* plants and its relates

4.2.3 Antimicrobial activity

Among ten prenylflavanones of *Sophora tomentosa* and *Sophora moorcroftiana*, sophoraflavanone G (**5**) showed the highest antimicrobial activity against three Gram-positive bacteria such as *Staphylococcus aureus* 6571, 8530 and 8531, followed by euchrestaflavanone A, sophoraflavanone H (**6**) and sophoraflavanone I (**7**). Sophoraflavanone G (**5**) also was effective against *Shigella dysenteria* 1, *Escherichia coli* R832 and *Vibrio cholerae* 865. Moreover, sophoraflavanone I (**7**) had the highest anti-*Helicobacter pylori*, followed by sophoraflavanone H (**6**) and sophoraflavanone G (**5**) (Shirataki et al., 2001a). Combined treatment of sophoraflavanone G (**5**) and ampicillin gave synergistic effects against *Streptococcus mutans*, *S. sanguinis, S. sobrinus, S. gordonii, Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Porphylomonas gingivalis*, whereas the combination treatment of sophoraflavanone G (**5**) and gentamicin was synergistic against *Streptococcus* sanguinis, *S. criceti, S. anginosus*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *P. gingivalis*. In particular, the minimum inhibitory concentrations/minimum bactericidal concentrations (MICs/MBCs) for all the bacteria could be reduced to 1/2-1/16 by their drug combinations.

Sophoraflavanone G (**5**) combined with other antibiotics might be microbiologically beneficial (Cha et al., 2007).

Eleven isoflavonoids were examined for their possible antimicrobial property against twelve known Gram-positive and Gram-negative sensitive bacteria. Daidzein (**3**) and calycosin failed to show antimicrobial activity and formononetin, genistein (**4**), biochanin A, irisolidone, 7,4'-dihydroxy-3'-methoxyisoflavone, licoisoflavone A and licoisoflavone B had moderate antimicrobial action. Sophoraisoflavone A and 6,8-diprenylgenistein showed higher antimicrobial activity *in vitro* against 214 strains of bacteria. Their MIC ranged from 25 to 200 mg/L in most strains. At the concentrations of 30 and 60 μg/mouse, sophoraisoflavone A and 6,8-diprenylgenistein significantly protected the mice from 50 median lethal dose (MLD) of a virulent strain of *Salmonella Typhimurium* (Dastidar SG et al., 2004).

4.2.4 Anti-HIV activity

Among ten prenylflavanones, sophoraflavanone G (**5**) (selectivity index (SI) showed weak anti-HIV activity, followed by euchrestaflavanone A (SI=3) and sophoraflavanone H (**6**) (SI=3). Prenylflavanones having either prenyl-, lavandulyl- or geranyl groups on A-ring and/or B-ring, and flavonostilbenes having stilbene (resveratrol) on B-ring showed anti-HIV activity. A good relationship between their anti-HIV activity and radical generation or O_2^- scavenging activity was observed. Eleven isoflavones failed to induce their anti-HIV activity (Shirataki et al., 2001a, 2001b).

4.2.5 Radical generation and radical O_2^- scavenging activity

Among ten prenylflavanones, 6-prenylnaringenin and sophoraflavanone G (**5**), sophoraflavanone H (**6**), sophoraflavanone I (**7**) and euchrestaflavanone A showed radical generation and O_2^- scavenging activity (Shirataki et al., 2001a). Sophoraflavanone G (**5**) and Kurarinone (**9**) protected renal epithelial LLC-PK(1) cells from 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH)-induced injury, possibly by their antioxidative activity (Piao et al., 2006). Prenylated chalcones such as kuraridin and kuraridinol scavenged DPPH radical. Five flavanones such as kushenol E, leachianone G, kurarinol (**8**), sophoraflavanone G (**5**), and kurarinone (**9**) inhibited *t*-BHP-induced NF-κB activation and exhibited significant antioxidant potentials against 2,2'-azino-*bis*-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), peroxynitrite (ONOO⁻), and reactive oxygen species (ROS). Prenylated chalcones or flavonol showed higher scavenging and inhibitory activities than prenylated flavanones. Additionally, the results showed that *Sophora flavescens* and its prenylated flavonoids could have their good anti-inflammatory activity, possibly due to their significant antioxidant activity (Jung et al., 2008).

Five compounds such as *trans*-hexadecyl ferulic acid, *cis*-octadecyl ferulic acid, *trans*-hexadecyl sinapic acid, (-)-4-hydroxy-3-methoxy-(6a*R*,11a*R*)-8,9-methylenedioxypterocarpan and desmethylanhydroicaritin exhibited their DPPH and ONOO⁻ scavenging activities (Jung et al., 2005). All isoflavones [daidzein (**3**), genistein (**4**)] and isoflavanones did not stimulate their NO production by mouse macrophage-like Raw264.7 cells, but almost completely inhibited their NO production by the LPS-activated Raw264.7 cells. Secundifloran and secundiflorol D most potently inhibited the NO production, but also efficiently scavenged the O_2^- and NO radicals.

The inhibition of macrophage NO production by these isoflavanones might, at least in part, be explained by their radical scavenging or reduction activity (Shirataki et al., 2004).

4.2.6 Enzyme inhibitory activity

Sophoflavescenol with a C-8 prenylated flavonol inhibited cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5), more efficiently than PDE3 and PDE4. Among twelve prenylated flavonoids with the resorcinol moiety, kuraridin (**10**), sophoraflavanone G (**5**), kurarinone (**9**) and norkurarinol inhibited the tyrosinase activity more effectively than kojic acid, a tyrosinase inhibitor. The substitution of a lavandulyl or hydroxylavandulyl group at the C-8 position and a methoxy or hydroxy group at the C-5 position (Fig. 13) may be required for their inhibitory effect (Son et al., 2003, Kim et al., 2003)
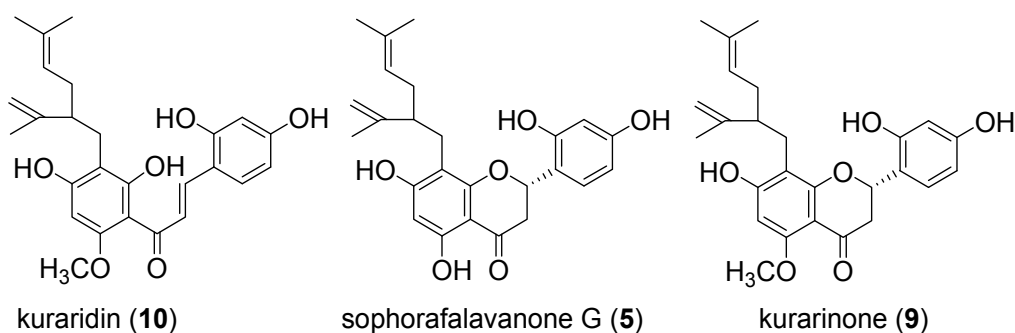

kuraridin (**10**)   sophorafalavanone G (**5**)   kurarinone (**9**)

Fig. 13. *Sophora flavescens* that inhibit tyrosinase activity

4.3 State in hot-water extract: Possible binding to other compounds

Neutral aqueous solutions in anthocyanins are unstable and quickly decolorized. The copigmentation reaction, first reported by Robinson, is a colour-stabilizing mechanism (Oszmianski et al., 2004) and regarded as significant factors for stabilizing the structure of anthocyanins (Marcovic et al., 2000). The copigmentation reaction occurs, when planar molecule interaction between anthocyanin and other pigments occurs to form the complex. The common copigment compounds are flavonoids such as rutin and quercetin (Bakowska et al., 2003), other polyphenols, amino acids and organic acids.

4.4 Site of action, signaling pathway, and receptor identification

It has been recently reported that baicalin, a flavone, induced the osteoblastic differentiation *via* the activation of Wnt/β-catenin signalling pathway (Guo et al, 2011). Apigenin induced cell growth retardation *via* leptin/leptin receptor pathway in human A549 adenocarcinoma cell line (Bruno et al., 2011).

4.5 Future direction

Flavonoids are distributed into virtually all parts of the plant, the root, heartwood, sapwood, bark, leaf, fruit and flower. Although various pharmacological and biological activities of flavonoids are reported, the reason why biosynthesis of prenylflavonoids is restricted in Leguminosae, Moraceae and a few species of plants is unclear. Further chemical and physiological investigations are needed to answer this question.

5. Saponins

Saponins are naturally occurring glycosides with the ability of forming a soapy lather when shaken with water, and classified as triterpene saponins or steroidal saponins on the basis of the structural features of the aglycone moieties. Triterpene saponins are found in a wide variety of dicotyledonous plants, but are rare in monocotyledons (Dinda et al., 2010). On the other hand, steroidal saponins are mainly distributed among a limited species of monocotyledonous plants (Mahato et al., 1982). Some marine organisms are known to produce saponins with unique chemical structures and biological properties (Minale et al., 1996).

Several important Chinese herbal medicines such as Astragalus Root, Licorice Root, Polygala Root, Ginseng, Bupleurum Root, Anemarrhena Rhizome and Ophiopogon Tuber contain saponins as the main secondary metabolites, which may be responsible for their specific pharmacological activities. Depending on the fundamental skeletons of the aglycone moieties, triterpene saponins have been classified into more than 10 types, oleanane-type, ursane-type, lupan-type, dammarane-type, and so on. As for steroidal saponins, they have been classified as being spirostan-type, furostan-type, or cholestane-type. Cardiac glycosides and pregnane glycosides are parts of steroidal glycosides, but are not usually included in the saponin category.

In this section, the current aspects of a potent cytotoxic saponin of a cholestane-type, 17α-hydroxy-16β-[(*O*-(2-*O*-*p*-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy)]cholest-5-en-22-one (OSW-1) (Fig. 14), are mainly described according to the concept of this Chapter.
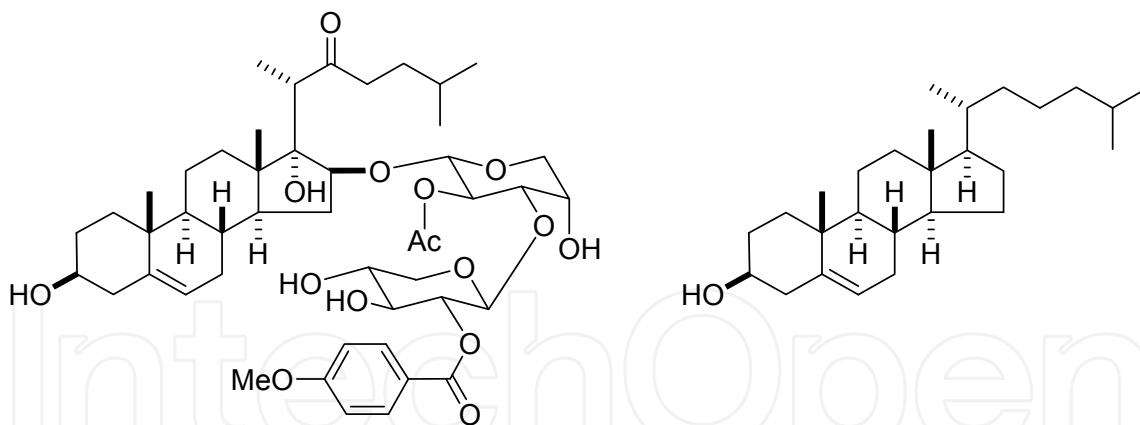

Fig. 14. Structures of OSW-1 (left) and cholesterol (right)

5.1 Yield and putative amounts in methanol and hot water extracts

Saponins can to be extracted with hot water. However, since saponins are structurally composed of a hydrophobic aglycone unit and a hydrophilic sugar moiety, methanol and a mixture of ethanol and water (4:1, v/v) allow saponins, including cardiac and pregnane glycosides with deoxysugar residues, to be exhaustively extracted from crude materials. OSW-1 was quantitatively extracted from a plant material using methanol. Pure ethanol fails to extract saponins with high polar properties such as glycyrrhizinic acid whose sugar moiety is composed of two glucuronic acid units (Kuroda et al., 2010).

5.2 Biological activity

5.2.1 Cytotoxic and antitumor activities of OSW-1

Isolation and identification of OSW-1: OSW-1 was isolated from the methanol extract of the bulbs of *Ornithogalum saundersiae*, a Liliaceae plant native to South Africa. The structure of OSW-1 was determined by conventional spectroscopic analysis and hydrolysis, and was revealed to be a cholestane-type saponin with an acylated sugar unit at the C-16β hydroxy group of the cholestane skeleton (Kubo et al., 1992).

Cytotoxic activities: OSW-1 exhibited significantly potent cytotoxic activities against various cultured malignant tumor cells such as mouse mastocarcinoma, human pulmonary adenocarcinoma, human pulmonary large cell carcinoma and human pulmonary squamous cell carcinoma, including adriamycin (ADM)-resistant P388 leukemia and camptothecin (CPT)-resistant P388; the activities are around 10-100 times more potent than those of the clinically used anticancer drugs, mitomycin C (MMC), ADM, cisplatin (CDDP), CPT and taxol (TAX). To the contrary, normal human pulmonary fibroblasts are little sensitive to OSW-1 (Mimaki et al., 1997).

Panel screening: Evaluation of OSW-1 in the Japanese Foundation for Cancer Research 38 cell-line assay (Yamori et al., 1999) showed that the mean concentration required to achieve GI_{50} against the panel cells tested was 5.6 nM. OSW-1 displayed differential cytotoxicities, with breast cancer, CNS cancer and lung cancer subpanel cell lines showing particular sensitivity, but with colon cancer, ovarian cancer, stomach cancer and prostate cancer subpanel cell lines being relatively resistant to it. The pattern of the differential cytotoxicities of OSW-1 was evaluated using the Compare Program and was revealed not to correlate with those shown by any of the other compounds, including currently used anticancer drugs; correlation coefficient values were less than 0.5. This indicates that OSW-1 must have a unique mode of action and the potentiality as the lead of a new anticancer agent.

In vivo evaluation: OSW-1 was not haemolytic in human erythrocytes at 100 μg/mL. In *in vivo* evaluations, OSW-1 was effective versus mouse P388 with an increased life span of 59% by one-time administration (i.p.) of 0.01 mg/kg. As for the response of Hep134 rat xenograft to OSW-1, administration of OSW-1 on the second day and the fourth day (each 0.04 mg/kg, i.p.) significantly reduced tumor growth compared to the non-treated group (unpublished data).

OSW-1 analogues: O. saundersiae yielded several OSW-1-related compounds, which were slightly different from OSW-1 in the structures of the aromatic acid moieties. A few more polar analogues having a glucosyl unit at C-4 of the terminal xylosyl moiety were also isolated and identified. Phytochemical studies of *Ornithogalum thyrsoides* and *Galtonia candicans*, which are taxonomically related to *O. saundersiae*, resulted in the isolation of a series of OSW-1 derivatives, possessing a glucosyl, diglucosyl, or triglucosyl unit at the C-3 hydroxy group of the aglycone moiety without exceptions (Mimaki, 2006). These OSW-1 analogues, together with those partially modified by hydrolysis and reduction, were useful for elucidating the structure-activity relationship (SAR) of OSW-1.

Total synthesis of OSW-1: Since the potentiality of OSW-1 as a new lead compound for a new anticancer agent was evident, OSW-1 has been an attractive synthetic target for organic

chemists. Furthermore, a number of OSW-1 analogues with modified acylated diglycosides, side-chains and/or steroidal nuclei have been obtained by means of chemical synthesis for SAR studies. Guo and Fuchs reported the first synthesis of the protected aglycone of OSW-1 (Guo & Fuchs, 1999). By employing the same approach, Deng and his co-workers established the first total synthesis of OSW-1 in 1999 (Deng et al., 1999). Yu and Jin reported a total synthesis of OSW-1 based on their own new strategy (Yu & Jin, 2001). Most recently, Xue and his co-workers have succeeded in the gram scale total synthesis of OSW-1 in 10 linear steps with an overall yield of 6.4%, starting from (+)-dehydroisoandrosterone (Xue et al., 2008).

Panel/cell line		$\log GI_{50}$	Panel/cell line		$\log GI_{50}$
Breast Cancer	HBC-4	-8.03	Melanoma	LOX-IMVI	-9.44
	BSY-1	-9.20			
	HBC-5	-8.73	Ovarian Cancer	OVCAR-3	-6.86
	MCF-7	-9.42		OVCAR-4	-8.27
	MDA-MB-231	-8.70		OVCAR-5	-8.27
				OVCAR-8	-6.00
CNS Cancer	U251	-9.51		SK-OV-3	-7.70
	SF-268	-7.48			
	SF-295	-10.00	Renal Cancer	RXF-631L	-8.79
	SF-539	-10.00		ACHN	-6.39
	SNB-75	-10.00			
	SNB-78	-9.31	Stomach Cancer	St-4	-8.06
				MKN1	-6.07
Colon Cancer	HCC2998	-7.42		MKN7	-7.26
	KM-12	-8.98		MKN28	-6.24
	HT-29	-8.24		MKN45	-6.00
	HCT-15	-8.17		MKN74	-9.30
	HCT-116	-8.48			
			Prostate Cancer	DU-145	-6.44
Lung Cancer	NCI-H23	-7.69		PC-3	-7.28
	NCI-H226	-7.15			
	NCI-H522	-9.01	Mean value		-8.25
	NCI-H460	-10.00			(GI_{50} 5.6 nM)
	A549	-8.76			
	DMS273	-10.00			
	DMS114	-9.20			

Table 3. The $\log GI_{50}$ values of OSW-1 against the 38 cell lines. The GI_{50} value is the concentration that yields 50% growth.

SAR of OSW-1: The aglycone of OSW-1 is a cholesterol (cholest-5-en-3β-ol) derivative with a β-hydroxy group at C-16, an α-hydroxy group at C-17 and a carbonyl group at C-22. The sugar moiety attached to C-16 of the aglycone was assigned as *O*-(2-*O*-*p*-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl. The acylated sugar moiety is essential for the exhibition of the potent cytotoxic activities of OSW-1 (Morzycki et al., 2004; Tang et al., 2007). Slight differences in the aromatic acid structure gave no effects on the activities (Mimaki et al., 1997), but the following modifications, 1) deacylation (Mimaki et al, 1997), 2) glucosylation at C-4 of the xylosyl unit (Kuroda et al., 2001), 3) removal of the C-4 hydroxy group of the arabinosyl unit (Tschamber et al., 2007), 4) change in the linkage position of the xylosyl unit from C-3 of the arabinosyl moiety to its C-4 (Zheng et al., 2010, 2011), significantly reduced its cytotoxicities.

Although replacement of the aglycone by disparate steroids led to inactive compounds (Ma et al., 2000, 2001a, 2001b), the steroidal aglycone moiety tolerate certain modifications without a significant loss in cytotoxic potency. The C-17α hydroxy group was shown to be of little importance for the cytotoxicities (Zheng et al., 2010, 2011). The C-22 carbonyl group was reported to be a pharmacophore requirement (Wojtkielewicz et al., 2007); however, a current SAR study concluded that it is not necessary for the cytotoxic activities (Zheng et al., 2011). A number of the side chain modified analogues of OSW-1 have been synthesized, and some C-23-oxa analogues have been found to be significantly cytotoxic (Deng et al., 2004; Shi et al., 2005). The steroidal A- and B-rings can be generally modified without a loss of cytotoxic potency, as was evident when the 3-O-glucopyranosyl- (Mimaki, 2006), 3-O-biotinyl- (Kang et al., 2009) and 5,6-dihydro- (Guan et al., 2011) OSW-1 derivatives were as active as the parent. However, the activity of a derivative with an aromatic A-ring was exceptionally much less than that of OSW-1 (Tschamber et al., 2007). The linkage of the acylated diglycoside to the C-16β hydroxy group of the aglycone is essential for the potent cytotoxicity (Ma et al., 2001b), except in the case of a few synthetic analogues (Guan et al., 2011; Zheng et al., 2011).

5.2.2 Other biological activities of OSW-1

At first, OSW-1 was isolated from a cyclic AMP phosphodiesterase inhibitor from *O. saundersiae* bulbs (Kubo et al., 1992). Tamura and his co-workers found that OSW-1 inhibited ovarian E_2 secretion. The decrease in the levels of ovarian steroid induced by OSW-1 was shown to be due to its direct inhibitory action on the gene expression of the steroidal enzyme and the proliferation of granulose cells in the ovary (Tamura et al., 1997).

5.2.3 Other cholestane-type saponins with cytotoxic activities

O. saundersiae bulbs produced a novel 24(23→22)*abeo*-cholestane glycoside named saundersioside B, which has a six-membered hemiacetal ring between C-16 and C-23, and a five-membered acetal ring between C-18 and C-20. Saundersioside B possesses an aromatic acid ester group at the sugar moiety attached to C-3 of the aglycone and inhibited HL-60 cell growth through induction of apoptosis (Kuroda et al., 1997).

Candicanoside A, isolated from *G. candicans*, has two epoxy functional groups between C-16 and C-23, and between C-18 and C-23 in its rearranged cholestane skeleton (Mimaki et al., 2000). A novel polyoxygenated 5β-cholestane glycoside, designated as galtonioside A, has also been isolated from *G. candicans* (Kuroda et al., 2000). These cholestane-type saponins also display potent cytotoxic activities against cultured tumor cells with differential cytotoxicities in panel screenings. The obtained cytotoxic profiles are related to that of OSW-1.

5.3 State in hot-water extract: Possible binding to other compounds

Although some spirostan-type saponins such as digitonin are characterized by the formation of complex with cholesterol, it has not been reported that cholestane-type saponins interact with other compounds. When OSW-1 is dissolved in water, methanol, or other alcohols and the solution is allowed to stand for a few days, OSW-1 gradually decomposes to afford deacyl derivatives.
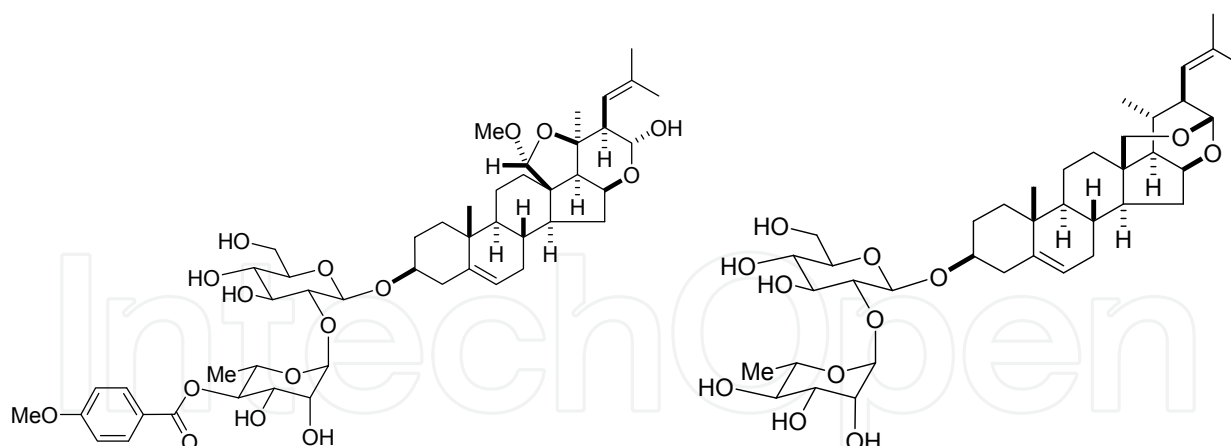
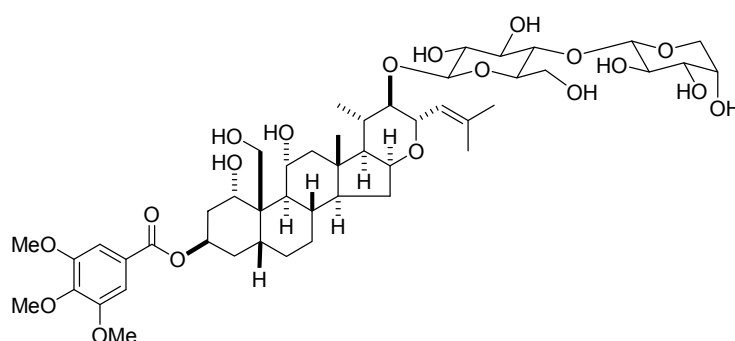Fig. 15. Structures of saundersioside B (left) and candicanoside A (right)

Fig. 16. Structure of galtonioside A

5.4 Site of action, signaling pathway, and receptor identification

The action mechanism of OSW-1 has been recently disclosed to damage the mitochondrial membrane and cristae in human leukemia and pancreatic cancer cells, leading to losses of transmembrane potential, increases in cytosolic calcium contents, and activation of calcium-dependent pathways. No anticancer compounds reported to date have this mechanism (Zhou et al., 2005). Furthermore, OSW-1 has been shown to induce apoptosis in mammalian cells through the mitochondrial pathway, involving the caspase-8-depending cleavage of Bcl-2 (Zhu et al., 2005).

5.5 Future directions

A number of crude drugs, including those used in Kampo prescriptions, are known to contain saponins. However, the pharmacological roles of saponins in Kampo prescriptions remain to be elucidated, except for glycyrrhizinic acid in Licorice Root. Manifestation of the specific pharmacological effects of each saponin will lead to providing evidence on the efficacy of Kampo medicines.

It is notable that *O. saundersiae*, an ornamental plant without a folkloric medicinal background, produces OSW-1, a cholestane-type steroidal saponin with high apparent potential for effectively treating some cancers that are resistant to available medicines. Thus, OSW-1 clearly warrants further chemical and biological investigations for its clinical applications.

6. Conclusion

This review summarizes unique biological properties of LCCs, tannins, flavonoids and saponins, suggesting their functionality as alternative medicines. LCCs showed broad and potent anti-viral activity and synergism with vitamin C. Since virus is one of major risk factor of oral cavity cancer (Sakagami; 2010a), anti-viral action of LCC may reduce the incidence of virus-triggered diseases such as cancer. The use of a genetic algorithm-kernel partial least squares algorithm combined with an artificial neural network (Jalai-Heravi, 2008) may be useful to predict the optimal structure of LCCs for the expression of biological activities. Identification of dectin-2 as LCC receptor awaits further confirmation with siRNA and gene overexpression experiments. Tannins are found to be relatively unstable in hot water, and easily decomposed and associated with serum albumin. Biological significance of such degradation products and associates remains to be investigated. Saponins with high polarity can to be extracted with hot water, and therefore should be present in many herbal extracts and Kampo medicines. The antitumor potentital of OSW-1 should be further pursued. .

7. Acknowledgment

8. References

Abe, M., Okamoto, K., Konno, K. & Sakagami, H. (1989). Induction of antiparasite activity by pine cone lignin-related substances. *In vivo* Vol.3, pp. 359-362.

Abou-Zaid, M.M., Lombardo, D.A., Kite, G.C., Grayer, R.J., & Veitch, N.C. (2001). Acylated flavone *C*-glycosides from *Cucumis sativus*. *Phyotochemistry* Vol.58. pp. 167-172.

Azuma, J-I. & Koshijima, T. (1988). Lignin-carbohydrate complexes from various sources. *Methods Enzymol* Vol.161. pp. 12-18.

Bakowska, A., Kucharska, A.Z. & Ozmianski, J. (2003). The effects of heating, UV and storage on stability of the anthocyanin-polyphenol copigment complex. *Food Chem* Vol.81. pp. 349-355.

Brown, G.D. & Gordon, S. (2001). Immune recognition. A new receptor for beta-glucans. *Nature* Vol.413(6851), pp. 36-37.

Bruno, A., Siena, L., Gerbino, S., Ferraro, M., Chanez, P., Giammanco, M., Gjomarkaj, M. & Pace, E. (2011). Apigenin affects leptin/leptin receptor pathway and induces cell apoptosis in lung adenocarcinoma cell line. *Eur J Cancer*, in press.

Cha, J.D., Jeong, M.R., Jeong, S.I. & Lee, K.Y. (2007). Antibacterial activity of sophoraflavanone G isolated from the roots of Sophora flavescens. *J Microbiol Biotechnol* Vol.17, pp.858-864.

Dastidar, S.G., Manna, A., Kumar, K.A., Mazumdar, K., Dutta, N.K., Chakrabarty, A.N., Motohashi, N. & Shirataki, Y. (2004). Studies on the antibacterial potentiality of isoflavones. *Int J Antimicrob Agents* Vo.23, pp. 99-102.

Davin, L.; Wang, H.B., Crowell A.L.,Bedgar, D.L., Martin, D.M., Sarkanen, S. & Lewis, N.G. (1997). Stereoselective biomolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. *Science* Vol.275, pp. 362-366.

Deng, S., Yu, B., Lou, Y. & Hui, Y. (1999). First total synthesis of an exceptionally potent antitumor saponin, OSW-1. *J Org Chem* Vo.64, pp. 202-208.

Deng, L., Wu, H., Yu, B., Jiang, M. & Wu, J. (2004). Synthesis of OSW-1 analogs with modified side chains and their antitumor activities. *Bioorg Med Chem Lett* Vo.14, pp. 2781-2785.

Dinda, B., Debnath, S., Mohanta, B.C. & Harigaya, Y. (2010). Naturally occurring triterpenoid saponins. *Chem Biodivers* Vo.7, pp. 2327-2580.

Emiliani, G., Fondi, M., Fani, R. & Gribaldo, S. (2009). A horizontal gene transfer at the origin of phenylpropanoid metabolism: a key adaptation of plants to land. *Biology Direct* Vol.4, 4 (https://www.biology-direct.com/content/4/1/7)

Fukai, T., Sakagami, H., Toguchi, M., Takayama, F., Iwakura, I., Atsumi, T., Ueha, T., Nakashima, H. & Nomura, T. (2000). Cytotoxic activity of low molecular weight polyphenols against human oral tumor cell lines. *Anticancer Res* Vol.20, pp. 2525-2536.

Fukuchi, K., Sakagami, H., Ikeda, M., Kawazoe, Y., Oh-hara, T., Konno, K., Ichikawa, S., Hata, N., Kondo, H. & Nonoyama, M. (1989a). Inhibition of herpes simplex virus infection by pine cone antitumor substances. *Anticancer Res* Vol.9, pp. 313-318.

Fukuchi, K., Sakagami, H., Okuda, T., Hatano, T., Tanuma, S., Kitajima, K., Inoue, Y., Inoue, S., Ichikawa, S., Nonoyama, M. & Konno, K. (1989b). Inhibition of herpes simplex virus infection by tannins and related compouneds. *Antiviral Res* Vol.11, pp. 285-298.

Gross, O., Gewies, A., Finger, K., Schäfer, M., Sparwasser, T., Peschel, C., Förster, I. & Ruland, J. (2006). Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* Vo.442(7103), pp. 651-656.

Guan, Y., Zheng, D., Zhou, L., Wang, H., Yan, Z., Wang, N., Chang, H., She, P. & Lei, P. (2011). Synthesis of 5(6)-dihydro-OSW-1 analogs bearing three kinds of disaccharides linking at 15-hydroxy and their antitumor activities. *Bioorg Med Chem Lett* Vol.21: pp. 2921-2924.

Guo, A.J.Y., Choi, R.C.Y., Cheung, A.W.H., Chen, V.P., Xu, S.L., Dong, T.T.X., Chen, J.J., & Tsim, K.W.K. (2011). Baicalin, a flavone, induces the differentiation of cultured osteoblasts: an action via the WNT/β-catenin signaling. *J Biol Chem* in press.

Guo, C. & Fuchs, P.L. (1998). The first synthesis of the aglycone on the potent anti-tumor steroidal saponin OSW-1. *Tetrahedron Lett* Vol.39, pp. 1099-1102.

Hanaoka, A., Sakgami, H. & Konno, K. (1989). Pine cone antitumor substances stimulate cytotoxic factor production in young mice, but not in aged or tumor-bearing mice. *Showa Univ J Med Sci*, Vol.1, pp. 57-63.

Harada, H., Sakagami, H., Konno, K., Sato, T., Osawa, N., Fujimaki, M. & Komatsu, N. (1988). Induction of antimicrobial activity by antitumor substances from pine cone extract of *Pinus parviflora* Sieb. et Zucc. *Anticancer Res,* Vol. 8, pp. 581-588

Harada, H., Sakagami, H., Nagata, K., Oh-hara, T., Kawazoe, Y., Ishihama, A., Hata, N., Misawa, Y., Terada, H. & Konno, K. (1991). Possible involvement of lignin structure in anti-influenza virus activity. *Antiviral Res* Vol.15, pp. 41-50.

Hatano, T., Hori, M., Hemingway, R., & Yoshida, T. (2003a). Size exclusion chromatographic analysis of polyphenols-serum albumin complexes. *Phytochemistry*, Vol.63, pp. 817-823.

Hatano, T., Kusuda, M., Hori, M., Shiota, S., Tsuchiya, T. & Yoshida, T. (2003b). Theasinensin A, a tea polyphenols formed from (-)-epigallocatechin gallate, suppresses antibiotic resistance of methicillin-resistant *Staphylococcus aureus*. *Plant Med*, Vol.69, pp. 984-989.

Hu, H., Chai, Y., Wang, L., Zhang, J., Lee, H.J., Kim, S.H. & Lü, J. (2009). Pentagalloylglucose induces autophagy and caspase-independent programmed cell deaths in human PC-3 and mouse TRAMP-C2 prostate cancer cells. *Mol Cancer Ther*, Vol.8, pp. 2833-2843.

Ichimura, T., Otake, T., Mori, H. & Maruyama, S. (1999). HIV-1 protease inhibition and anti-HIV effect of natural and synthetic water-soluble lignin-like substances. *Biosci Biotechnol Biochem*, Vol.63, pp. 2202-2024.

Ito, H., Iguchi, A. & Hatano, T. (2008). Identification of urinary and intestinal bacterial metabolites of ellagitannin geraniin in rats. *J Agric Food Chem*, Vol.56, pp. 393-400.

Jalali-Heravi, M. (2008). Neural networks in analytical chemistry. *Methods Mol Biol*, Vol.458, pp. 81-121.

Joo, S.S., Kang, H.C., Lee, M.W., Choi, Y.W. & Lee, D.I. (2003). Inhibition of IL-1β and IL-6 in osteoblast-like cell by isoflavones extracted from Sophorae fructus. *Arch Pharm Res*, Vol.26, pp. 1029-1035.

Jung, H.J., Kang, S.S., Woo, J.J. & Choi, J.S. (2005). A new lavandulylated flavonoid with free radical and ONOO- scavenging activities from Sophora flavescens . *Arch Pharm Res*, Vol.28, pp. 1333-1336.

Jung, H.A., Jeong, D.M., Chung, H.Y., Lim, H.A., Kim, J.Y., Yoon, N.Y. & Choi, J.S. (2008). Re-evaluation of the antioxidant prenylated flavonoids from the roots of Sophora flavescens. *Biol Pharm Bull*, Vol.31, pp. 908-915.

Kang, Y., Lou, C., Ahmed, K.B., Huang, P. & Jin, Z. (2009). Synthesis of biotinylated OSW-1. *Bioorg Med Chem Lett*, Vol.19, pp. 5166-5168.

Kubo, S., Mimaki, Y., Terao, M., Sashida Y, Nikaido T & Ohmoto, T (1992): Acylated cholestane glycosides from the bulbs of *Ornithogalum saundersiae*. *Phytochemistry*, Vol.31, pp. 3969-3973.

Kawano, M., Sakagami, H., Satoh, K., Shioda, S., Kanamoto, T., Terakubo, S., Nakashima, H. & Makino, T. (2010a). Lignin-like activity of *Lentinus edodes* mycelia Extract (LEM). *In Vivo*, Vol.24, pp. 543-552.

Kay, R.M. (1982). Dietary fiber. *J Lipid Res*, Vol.23, pp. 221-242.

Kim, S.J., Son, K.H., Chang, H.W., Kang, S.S. & Kim, H.P. (2003). Tyrosinase inhibiting prenylated flavonoids from Sophora flavescens. *Biol Pharm Bull*, Vol.26, pp. 1348-1350.

Koizumi, N., Sakagami, H., Utsumi, A., Fujinaga, S., Takeda, M., Asano, K., Sugawara, I., Ichikawa, S., Kondo, H., Mori, S., Miyatake, K., Nakano, Y., Nakashima, H., Murakami, T., Miyano, N. & Yamamoto, N. (1993). Anti-HIV (human immunodeficiency virus) activity of sulfated paramylon. *Antiviral Res*, Vol.21, pp.1-14.

Kuo, P.T., Lin, T.P., Liu, L.C., Huang, C.H., Lin, J.K., Kao, J.Y. & Way, T.T. (2009). Penta-*O*-galloyl-beta-D-glucose suppresss prostate cancer bone metastasis by

transcriptionally repressing EGF-induced MMP-9 expression. *J Agric Food Chem*, Vol.57, pp3331-3339.

Kuroda, M., Mimaki, Y., Sashida, Y., Hirano, T., Oka, K., Dobashi, A., Li, H.Y. & Harada, N. (1997). Novel cholestane glycosides from the bulbs *Ornithogalum saundersiae* and their cytostatic activity on leukemia HL-60 and MOLT-4 cells. *Tetrahedron*, Vol.53, pp. 11549-11562.

Kuroda, M., Mimaki, Y., Sashida, Y., Yamori, T. & Tsuruo ,T. (2000). Galtonioside A, a novel cytotoxic cholestane glycoside from *Galtonia candicans. Tetrahedron Lett,* Vol.41, pp. 251-255.

Kuroda, M., Mimaki, Y., Yokosuka, A., Sashida, Y. & Beutler, J.A. (2001). Cytotoxic cholestane glycosides from the bulbs of *Ornithogalum saundersiae. J Nat Prod.* Vol.64, pp. 88-91.

Kuroda, M., Mimaki, Y., Honda, S., Tanaka, H., Yokota, S. & Mae, T. (2010). Phenolics from *Glycyrrhiza glabra* roots and their PPAR-γ ligand-binding activity. *Bioorg Med Chem*, Vol.18, pp. 962-970.

Kushida, T., Makino, T., Tomomura, M., Tomomura, A. & Sakagami, H. (2011). Enhancement of dectin-2 gene expression by lignin-carbohydrate complex from Lendinus edodes extract (LEM) in mouse macrophage-like cell line. *Anticancer Res*, Vol.31, pp. 1241-1248

Kusuda, M., Inada, K., Ogawa, T., Yoshida, T., Shiota, S., Tsuchiya, T. & Hatano, T. (2006). Polyphenolic constituents of *Zanthoxylum piperitum* fruit and the antibacterial effects of its polymeric proanthocyanidins on methicillin-resistant *Staphylococcus aureus. Biosc Biotechnol Bochem*, Vol.70, pp. 1423-1431.

Lai, P.K., Donovan, J., Takayama, H., Sakagami, H., Tanaka, A., Konno, K. & Nonoyama, M. (1990). Modification of human immunodeficiency viral replication by pine cone extacts. *AIDS Res Human Retroviruses,* Vol.6, pp. 205-217

Lai, P.K., Oh-hara, T., Tamura, Y., Kawazoe, Y., Konno, K., Sakagami, H., Tanaka, A. & Nonoyama, M. (1992). Polymeric phenylpropenoids are the active components in the pine cone extract that inhibit the replication of type-1 human immunodeficiency virus *in vitro. J Gen Appl Microbiol*, Vol.38, pp. 303-323.

Lewis, N.G. & Yamamoto, E. (1990). Lignin. Occurrence, biogenesis and biodegradation. *Ann Rev Plant Physiol Plant Nol Biol*, Vol.41, pp. 455-496.

López, B.S.G., Yamamoto, M., Utsumi, K., Aratsu, C. & Sakagami, H. (2009). Clinical pilot study of lignin-ascorbic acid combination treatment of herpes simplex virus. *In Vivo*, Vol.23, pp. 1011-1016, 2009

Ma, X., Yu, B., Hui, Y., Xiao, D. & Ding, J. (2000). Synthesis of glycosides bearing the disaccharide of OSW-1 or its 1→4-linked analogue and their antitumor activities. *Carbohydr Res*. Vol.329, pp. 495-505.

Ma, X., Yu, B., Hui, Y., Miao, Z. & Ding, J. (2001a). Synthesis of steroidal glycosides bearing the disaccharide moiety of OSW-1 and their antitumor activities. *Carbohydr Res*, Vol.334, pp. 159-164.

Ma, X., Yu, B., Hui, Y., Miao, Z. & Ding, J. (2001b). Synthesis of OSW-1 analogues and a dimer and their antitumor activities. *Bioorg Med Chem Lett*. Vol.11, pp. 2153-2156.

Mahato, S.B., Ganguly, A.N. & Sahu, N.P. (1985). Steroid saponins. *Phytochemistry*, Vol.21, pp. 959-978.

Manabe, H., Sakagami, H., Ishizone, H., Kusano, H., Fujimaki, M., Wada, C., Komatsu, N., Nakashima, H., Murakami, T. & Yamamoto, N. (1992). Effects of Catuaba extracts on microbial and HIV infection. *In Vivo*, Vol.6, pp. 161-166.

Marcovic, J.M., Petranovic, N.A. & Baranac, J.M. (2000): A spectrophotometric study of the copigmentation of malvin with caffeic and ferulic acid. *J Agric Food Chem*, Vol.48, pp. 5530-5536.

Martens, S. & Mithöfer, A. (2005). Flavones and flavone systhases. *Phytochemistry*, Vol.66, pp. 2399-2407.

Matsuta, T., Sakagami, H., Satoh, K., Kanamoto, T., Terakubo, S., Nakashima, H., Kitajima, M., Oizumi, H. & Oizumi T. (2011): Biological activity of luteolin glycosides and tricin from *Sasa senanensis* Rehder. *In Vivo*, Vol. 25, pp. 757-762.

McGreal, E.P., Rosas, M., Brown, G.D., Zamze, S., Wong, S.Y., Gordon, S., Martinez-Pomares, L. & Taylor, P.R. (2006). The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology*, Vol.16(5), pp. 422-430.

Mimaki, Y., Kuroda, M., Kameyama, A., Sashida, Y., Hirano, T., Oka, K., Maekawa, R., Wada, T., Sugita, K. & Beutler, J.A. (1997). Cholestane glycosides with potent cytostatic activities on various tumor cells from *Ornithogalum saundersiae* bulbs. *Bioorg Med Chem Lett*, Vol.7, pp. 633-636.

Mimak,i Y., Kuroda, M., Sashida, Y., Yamori, T. & Tsuruo, T. (2000). Candicanoside A, a novel cytotoxic rearranged cholestane glycoside from *Galtonia candicans*. *Helv Chim Acta*, Vol. 83, pp. 2698-2704.

Mimaki, Y. (2006). Structures and biological activities of plant glycosides: cholestane glycosides from *Ornithogalum saundersiae*, *O. thyrsoides* and *Galtonia candicans*, and their cytotoxic and antitumor activities. *Nat Prod Commun*, Vol.1, pp. 247-253.

Minale, L., Iorizzi, M., Palagiano, E. & Riccio, R. (1996). Steroid and triterpenoid oligoglycosides of marine origin. *Adv Exp Med Biol*, Vol.404, pp. 335-356.

Mohamed, A. A. O., Taniguchi, S., Yoshimura, M., Yoshida, T., Kishino, K., Sakagami, H. & Hatano, T. (2010). Hydrolyzable tannins of tamaricaceous plants. III. Hellinoyl- and macrocyclic-type ellagitannins from *Tamarix nilotica*. *J Nat Prod*, Vol.73, pp. 870-879.

Morzycki, J.W., Wojtkielewicz, A. & Wołczyński, S. (2004). Synthesis of analogues of a potent antitumor saponin OSW-1. *Bioorg Med Chem Lett*, Vol.14, pp. 3323-3326.

Nagata, K., Sakagami, H., Harada, H., Nonoyama, M., Ishihara, A. & Konno, K. (1990). Inhibition of influenza virus infection by pine cone antitumor substances. *Antiviral Res*, Vol.13, pp. 11-22.

Nakajima, Y., Yun, Y. S. & Kunugi, A. (2003). Six new flavonolignans from *Sasa veitchii* (Carr.) Rehder. *Tetrahedron*, Vol.59, pp. 8011-8015.

Nakane, A., Minagawa, T. & Kato, K. (1988). Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. *Infect Immun*, Vol.56, pp. 2503-2506.

Nakashima, H., Murakami, T., Yamamoto, N., Naoe, T., Kawazoe, Y., Konno, K. & Sakagami, H. (1992a). Lignified materials as medicinal resources. V. Anti-HIV (human immunodeficiency virus) activity of some synthetic lignins. *Chem Pharm Bull*, Vol.40, pp. 2102-2105

Nakashima, H., Murakami, T., Yamamoto, N., Sakagami, H., Tanuma, S., Hatano, T., Yoshida, T. & Okuda, T. (1992b). Inhibition of human immunodeficiency viral replication by tannins and related compounds. *Antiviral Res,* Vol.18, pp. 91-103

Nozaki, A., Kimura, T., Ito, H. & Hatano, T. (2009). Interaction of polyphenolic metabolites with human serum albumin: a circular dichroism study. *Chem Pharm Bull*, Vol.57, pp. 1019-1023.

Oh-hara, T., Sakagami, H., Kawazoe, Y., Kaiya, T., Komatsu, N., Ohsawa, N., Fujimaki, M., Tanuma, S. & Konno, K. (1990). Antimicrobial spectrum of lignin-related pine cone extracts of *Pinus parvilofra* Sieb. et Zucc. *In Vivo*, Vol.4, pp. 7-12.

Okuda, T., Mori, K. & Hatano, T. (1980). The distribution of geraniin and mallotusinic acid in the order Geraniales. *Phytochemistry*, Vol.19, pp. 547-551.

Okuda, T,, Hatano, T., Agata, I. & Nishibe, S. (1986). The constituents of tannic activities in labiatae plants. I. Rosmarinic acid from labiatae plants in Japan. *Yakugaku Zasshi,* Vol.106, pp. 1108-1111.

Oszmianski, J., Bakowska, A. & Piacente, S. (2004). Thermodynamic characteristics of copigmentation reaction of acylated anthocyanin isolated from blue flowers of *Scutellaria baicalensis* Georgi with copigments. *J Sci Food and Agric*, Vol.84, pp. 1500-1506.

Park, H., Lim, J.H., Kim, H.J., Choi, H.J. & Lee, I. (2007). Antioxidant flavone glycoside from the leaves of *Sasa borealis*. *Arch Pham Res*, Vol. 30, pp. 161-166.

Piao, X,L,, Piao, X.S., Kim, S.W., Park, J.H., Kim, H.Y. & Cai, SQ. (2006) .Identification and characterization of antioxidants from Sophora flavescens. *Biol Pharm Bull*, Vol.29, pp. 1911-1915.

Raghuraman, A,, Tiwari, V., Zhao, Q., Shukla, D., Debnath, A.K. & Desai, U.R. (2007). Viral inhibition studies on sulfated lignin, a chemically modified biopolymer and a potential mimic of heparin sulfate. *Biomacromolecules*, Vol.8, pp. 1759-1763.

Saijo, S,, Ikeda, S., Yamabe, K., Kakuta, S., Ishigame, H., Akitsu, A., Fujikado, N., Kusaka, T., Kubo, S., Chung, S.H., Komatsu, R., Miura, N., Adachi, Y., Ohno, N., Shibuya, K., Yamamoto, N., Kawakami, K., Yamasaki, S., Saito, T., Akira, S. & Iwakura, Y. (2010). Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. *Immunity*, Vol.32(5), Vol. 681-691.

Sakagami, H., Ikeda, M., Unten, S., Takeda, K., Murayama, J., Hamada, A., Kimura, K., Komatsu, N. & Konno, K. (1987). Antitumor activity of polysaccharide fractions from pine cone extract of *Pinus parviflora* Sieb. et Zucc. *Anticancer Res*, Vol7, pp. 1153-1160.

Sakagami, H., Kohno, S., Tanuma, S. & Kawazoe, Y. (1990a). Induction of cytotoxic factor in mice by lignified materials combined with OK-432 (Picibanil). *In Vivo*, Vol.4, pp. 371-376.

Sakagami, H., Nagata, K., Ishihama, A., Oh-hara, T. & Kawazoe, Y. (1990b). Anti-influenza virus activity of synthetically polymerized phenylpropenoids. *Biochem Biophys Res Commun*, Vol.172, pp. 1267-1272.

Sakagami, H., Kuroiwa, Y., Takeda, M., Ota, H., Kazama, K., Naoe, T., Kawazoe, Y., Ichikawa, S., Kondo, H., Yokokura, T. & Shikita, M. (1992a). Distribution of TNF endogenously induced by various immunopotentiators and *Lactobacillus casei* in mice. *In Vivo*, Vol.6, pp. 247-254

Sakagami, H., Takeda, M., Kawazoe, Y., Nagata, K., Ishihama, A., Ueda, M. & Yamazaki, S. (1992b). Anti-influenza virus activity of a lignin fraction from cone of *Pinus parviflora* Sieb. et Zucc. *In Vivo*, Vol.6, pp. 491-496.

Sakagami, H., Kuribayashi, N., Iida, M., Hagiwara, T., Takahashi, H., Yoshida, H., Shiota, F., Ohata, H., Momose, K. & Takeda, M. (1996a). The requirement for and mobilization of calcium during induction by sodium ascorbate and by hydrogen peroxide of cell death. *Life Sciences*, Vol.58, pp. 1131-1138.

Sakagami, H., Satoh, K., Ohata, H., Takahashi, H., Yoshida, H., Iida, M., Kuribayashi, N., Sakagami, T., Momose, K. & Takeda, M. (1996b). Relationship between ascorbyl radical intensity and apoptosis-inducing activity. *Anticancer Res*, Vol.16, pp. 2635-2644.

Sakagami, H., Satoh, K., Aiuchi, T., Nakaya, K. & Takeda, M. (1997). Stimulation of ascorbate-induced hypoxia by lignin. *Anticancer Res*, Vol.17, pp. 1213-1216.

Sakagami, H., Jiang, Y., Kusama, K., Atsumi, T., Ueha, T., Toguchi, M., Iwakura, I., Satoh, K., Ito, H., Hatano, T. & Yoshida, T. (2000a) Cytotoxic activity of hydrolysable tannins against human oral tumor cell lines – A possible mechanism. *Phytomedicine*, Vol.7, pp. 39-47.

Sakagami, H., Satoh, K., Hakeda, Y. & Kumegawa, M. (2000b). Apoptosis-inducing activity of vitamin C and vitamin K. *Cell Mol Biol*, Vol.46, pp. 129-143.

Sakagami, H., Hashimoto, K., Suzuki, S., Ogiwara, T., Satoh, K., Ito, H., Hatano, T., Yoshida, T. & Fujisawa, S. (2005). Molecular requirement of lignin for expression of unique biological activity. *Phytochemistry*, Vol.66 (17), pp. 2107-2119.

Sakagami, H., Asano, K., Satoh, K., Takahashi, K., Kobayashi, M., Koga, N., Takahashi, H., Tachikawa, R., Tashiro, T., Hasegawa, A., Kurihara, K., Ikarashi, T., Kanamoto, T., Terakubo, S., Nakashima, H., Watanabe, S. & Nakamura, W. (2007). Anti-stress, anti-HIV and vitamin C-synergized radical scavenging activity of mulberry juice fractions. *In Vivo*, Vol.21, pp. 499-506

Sakagami, H.. Satoh, K., Fukamachi, H., Ikarashi, T., Simizu, A., Yano, K., Kanamoto, T., Terakubo, S., Nakashima, H., Hasegawa, H., Nomura, A., Utsumi, K., Yamamoto, M., Maeda, Y. & Osawa, K. (2008). Anti-HIV and vitamin C-synergized radical scavenging activity of cacao husk lignin fractions. *In Vivo*, Vol.22, pp. 327-33

Sakagami, H., Kushida, T., Oizumi, T., Nakashima, H. & Makino, T. (2010a). Distribution of lignin carbohydrate complex in plant kingdom and its functionality as alternative medicine. *Pharmacology & Therapeutics*, Vol.128, pp. 91-105.

Sakagami, H., Zhou, L., Kawano, M., Thet, M.M., Takana, S., Machino, M., Amano, S., Kuroshita, R., Watanabe, S., Chu, Q., Wang, Q.T., Kanamoto, T., Terakubo, S., Nakashima, H., Sekine, K., Shirataki, Y., Hao, Z.C., Uesawa, Y., Mohri, K., Kitajima, M., Oizumi, H. & Oizumi, T. (2010b). Multiple biological complex of alkaline extract of the leaves of *Sasa senanensis* Rehder. *In Vivo*, Vol.24, pp. 735-744.

Sakagami, H., Kawano, M., Thet, M.M., Hashimoto, K., Satoh, K., Kanamoto, T., Terakubo, S., Nakashima, H., Haishima, Y., Maeda, Y. & Sakurai, K. (2011). Anti-HIV and immunomodulation activities of cacao mass lignin carbohydrate complex. *In Vivo*, Vol.25, pp. 229-236.

Sakagami, H. & Watanabe, S. (2011). Beneficial effects of mulberry on human health. In: Phytotherapeutics and Human Health: Pharmacological and Molecular Aspects, ed. Farooqui AA, in press, Nova Science Publishers, Inc, Hauppauge, NY.

Satoh, K., Ida, Y., Ishihara, M. & Sakagami, H. (1999). Interaction between sodium ascorbate and polyphenols. *Anticancer Res*, Vol.19, pp. 4177-4186.

Sharma, S.D., Meeran, S.M. & Katiyar, S.K. (2010). Proanthocyanidins inhibit in vitro and in vivo growth of human non-small cell lung cancer cells by inhibiting the prostaglandin E2 and prostaglandin E2 receptors. *Mol Cancer Ther*, Vol.9 (3), pp.569-580.

Shi, B., Tang, P., Hu, X., Liu, J.O. & Yu, B. (2005). OSW saponins: Facile synthesis toward a new type of structures with potent antitumor activities. *J Org Chem*, Vol.70, pp. 10354-10367.

Shimizu, M., Shiota, S., Mizushima, T., Ito, H., Hatano, T., Yoshida, T. & Tsuchiya, T. (2001). Marked potentiation of activity of β-lactams against methicillin-resistant *Staphylococcus aureus* by corilagin. A*ntimicrob Agents Chemother*, Vol.45, pp. 3198-3201.

Shiota, S., Shimizu, M., Mizushima, T., Ito, H., Hatano, T., Yoshida, T. & Tsuchiya, T. (1999). Marked reduction in the minimum inhibitory concentration (MIC) of β-lactams in methicillin-resistant *Staphylococcus aureus* produced by epicatechin gallate, and ingredient of green tea (*Camellia sinensis*). *Biol Pharm Bull*, Vol.22, pp. 1388-1390.

Shiota, S., Shimizu, M., Mizusima, T., Ito, H., Hatano, T., Yoshida, T. & Tsuchiya, T. (2000). Restoration of effectiveness of β-lactams on methicillin-resistant *Staphylococcus aureus* by tellimagrandin I from rose red. *FEMS Microbiol Lett*, Vol.185, pp. 135-138.

Shirataki, Y., Motohashi, N., Tani, S., Sakagami, H., Satoh, K., Nakashima, H., Mahapatra, S.K., Ganguly, K., Dastidar, S.G. & Chakrabarty, A.N. (2001a). In Vitro Biological Activity of Prenylflavanones. *Anticancer Res*, Vol.21, pp.275-280.

Shirataki, Y., Tani, S., Sakagami, H., Satoh, K., Nakashima, H., Gotoh, K. & Motohashi, N. (2001b). :Relationship between cytotoxic activity and tadical intensity of isoflavones from Sophora Species. *Anticancer Res*, Vol.21, pp.2643-2648.

Shirataki, Y., Wakae, M., Yamamoto, Y., Hashimoto, K., Satoh, K., Ishihara, M., Kikuchi, H., Nishikawa, H., Minagawa, K., Motohashi, N. & Sakagami, H. (2004). Cytotoxicty and radical modulating activity of isoflavones and isoflavanones from Sophora Species. *Anticancer Res*, Vol.24, pp. 14811488.

Son, J.K., Park, J.S., Kim, J.A., Kim, Y., Chung, S.R. & Lee, S.H. (2003). Prenylated flavonoids from the roots of Sophora flavescens with tyrosinase inhibitory activity. *Planta Med*, Vol.69, pp. 559-561.

Suzuki, H., Iiyama, K., Yoshida, O., Yamazaki, S., Yamamoto, N. & Toda, S. (1990). Structural characterization of the immunoreactive and antiviral water-solubilized lignin in an extract of the culture medium of *Lentinum edodes* Mycelia (LEM). *Agric Biol Chem*, Vol.54, pp.. 479-487

Tamura, K., Honda, H., Mimaki, Y., Sashida, Y. & Kogo, H. (1997). Inhibitory effect of a new steroidal saponin, OSW-1, on ovarian functions in rats. *Br J Pharmacol*, Vol.121, pp. 1796-1802.

Taniguchi, S., Kuroda, K., Doi, K., Tanabe, M., Shibata, T., Yoshida, T. & Hatano, T. (2008). Dimeric flavans from gambir and their structural correlations with (+)-catechin. *Heterocycles*, Vol.76, pp. 1171-1180.

Tang, P., Mamdani, F., Hu, X., Liu, J.O. & Yu, B. (2007). Synthesis of OSW saponin analogs with modified sugar residues and their antiproliferative activities. *Bioorg Med Chem Lett*, Vol.17, pp. 1003-1007.

Thakkar, J.N., Tiwari, V. & Dessai, U.R. (2010). Nonsulfated, cinnamic acid-based lignins are potent antagonists of HSV-1 entry into cells. *Biomacromolecules*, Vol.11, pp. 1412-1416.

Tschamber, T., Adam, S., Matsuya, Y., Masuda, S., Ohsawa, N., Maruyama, S., Kamoshita, K., Nemoto, H. & Eustache, J. (2007). New analogues of the potent cytotoxic saponin OSW-1. *Bioorg Med Chem Lett*, Vol.17, pp. 5101-5106.

Vautier, S., Sousa Mda, G. & Brown, G.D. (2010). C-type lectins, fungi and Th17 responses. *Cytokine Growth Factor Rev*, Vol.21(6), pp. 405-412.

Watanabe, M. (1999). Antioxidative phenolic compounds from Japanese barnyard millet (Echinochloa utilis) grauns. *J Agric Food Chem*, Vol.47, pp. 4500-4505.

Watanabe, M. & Ito, M. (2002). Changes in antioxidative activity and flavone comosition of extracts from aerial parts of buckwheat during growth period. *Nippon Shokuhin Kagaku Kogaku Kaishi* Vol.49, pp. 119-125 (in Japanese).

Wojtkielewicz, A., Długosz, M., Maj, J., Morzycki, J.W., Nowakowski, M., Renkiewicz, J., Strnad, M., Swaczynová, J., Wilczewska, A.Z. & Wójcik, J. (2007). New analogues of the potent cytotoxic saponin OSW-1. *J Med Chem*, Vol.50, pp. 3667-3673.

Xue, J., Liu, P., Pan, Y. & Guo, Z. (2008). A total synthesis of OSW-1. *J Org Chem*, 73: pp. 157-161.

Yamori, T., Matsunaga, A., Sato, S., Yamazaki, K., Komi, A., Ishizu, K., Mita, I,. Edatsugi, H., Matsuba, Y., Takezawa, K., Nakanishi, O., Kohno, H., Nakajima, Y., Komatsu, H., Andoh, T. & Tsuruo, T. (1999). Potent antitumor activity of MS-247, a novel DNA minor groove binder, evaluated by an *in vitro* and *in vivo* human cancer cell line panel. *Cancer Res*, Vol.59, pp. 4042-4049.

Yoshida, T., Hatano, T., Kuwajima, T. & Okuda, T. (1992). Oligomeric hydrolyzable tannins – their ^{1}H NMR spectra and partial degradation. *Heterocycles*, Vol.33, pp.463-482.

Yu, W. & Jin, Z. (2001). A new strategy for the stereoselective introduction of steroid side chain via α-alkoxy vinyl cuprates: Total synthesis of a highly potent antitumor natural product OSW-1. *J Am Chem Soc*, Vol.123, pp. 3369-3370.

Zhang, Y., But, P.P., Ooi, V.E., Xu, H.X., Delaney, G.D., Lee, S.H. & Lee, S.F. (2007). Chemical properties, mode of action, and in vivo anti-herpes activities of a lignin-carbohydrate complex from Prunella vulgaris. *Antiviral Res*, Vol.75, pp. 242-249.

Zheng, D., Zhou, L., Guan, Y., Chen, X., Zhou, W., Chen, X. & Lei, P. (2010). Synthesis of cholestane glycosides bearing OSW-1 disaccharide or its 1→4-linked analogue and their antitumor activities. *Bioorg Med Chem Lett*, Vol.20, pp. 5439-5442.

Zheng, D., Guan, Y., Chen, X., Xu, Y., Chen, X. & Lei, P. (2011). Synthesis of cholestane saponins as mimics of OSW-1 and their cytotoxic activities. *Bioorg Med Chem Lett*, Vol.21, pp. 3257-3260.

Zhou, Y., Garcia-Prieto, C., Carney, D.A., Xu, R.H., Pelicano, H., Kang, Y., Yu, W., Lou, C., Kondo, S., Liu, J., Harris, D.M., Estrov, Z., Keating, M.J., Jin, Z. & Huang, P. (2005). OSW-1: a Natural compound with potent anticancer activity and a novel mechanism of action. *J Natl Cancer Inst,* Vol.97, pp. 1781-1785.

Zhu, J., Xiong, L., Yu, B. & Wu, J. (2005). Apoptosis induced by a new member of saponin
        family is mediated through caspase-8-dependent cleavage of Bcl-2. *Mol Pharmacol,*
        Vol.68, pp. 1831-1838.
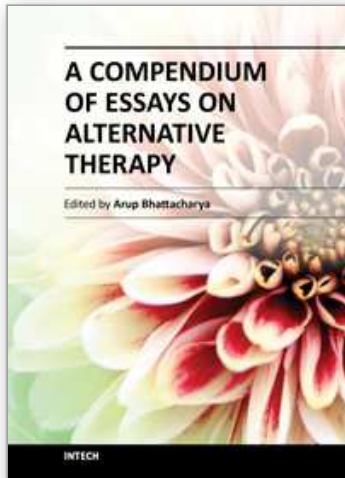
A Compendium of Essays on Alternative Therapy

Edited by Dr. Arup Bhattacharya

A Compendium of Essays on Alternative Therapy is aimed at both conventional and alternate therapy practitioners, besides serving as an educational tool for students and lay persons on the progress made in the field. While this resource is not all-inclusive, it does reflect the current theories from different international experts in the field. This will hopefully stimulate more research initiatives, funding, and critical insight in the already increasing demand for alternate therapies that has been evidenced worldwide.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Hiroshi Sakagami, Tatsuya Kushida, Toru Makino, Tsutomu Hatano, Yoshiaki Shirataki, Tomohiko Matsuta, Yukiko Matsuo and Yoshihiro Mimaki (2012). Functional Analysis of Natural Polyphenols and Saponins as Alternative Medicines, A Compendium of Essays on Alternative Therapy, Dr. Arup Bhattacharya (Ed.), ISBN: 978-953-307-863-2, InTech, Available from: http://www.intechopen.com/books/a-compendium-of-essays-on-alternative-therapy/functional-analysis-of-natural-polyphenols-and-saponins-as-alternative-medicines

INTECH
open science | open minds