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Critical Review

Biological Activities and Possible Dental Application of Three Major Groups of Polyphenols

Hiroshi Sakagami[1,*]

[1]*Division of Pharmacology, Department of Diagnostic and Therapeutic Sciences, Meikai University School of Dentistry, Sakado 350-0283, Japan*

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Abstract. The present article reviewed the biological activities and possible dental application of three major polyphenols, i.e., lignin-carbohydrate complexes, tannins, and flavonoids, citing mostly our in vitro studies together with those from other groups. All these polyphenols showed much lower tumor-selective cytotoxicity against oral squamous cell carcinoma cells vs. normal oral cells (gingival fibroblast, pulp cell, periodontal ligament fibroblast), in comparison to popular chemotherapeutic antitumor drugs. Several compounds showing higher tumor-selectivity did not induce internucleosomal DNA fragmentation, a biochemical hallmark of apoptosis, in oral carcinoma cell lines. Lignin-carbohydrate complex protected the cells from the cytopathic effects of HIV infection and UV irradiation more efficiently than other polyphenols. Limited digestion of lignin-carbohydrate complex suggests that the lignin moiety is involved in the prominent anti-HIV activity, whereas the carbohydrate moiety may function in immunopotentiating activity through a cell surface receptor. Alkaline extract of plant leaf, which contains higher amounts of lignin-carbohydrate complex, showed potent anti-inflammatory action against IL-1β-stimulated human gingival fibroblasts. Local application of lignin-carbohydrate complex through oral mucosa is recommended, considering its poor intestinal absorption.

Keywords: polyphenol, oral carcinoma, gingivitis, viral infection, anti-UV activity

1. Introduction

Tannins, flavonoids, and lignin-carbohydrate complexes are three major classes of polyphenols in the land-plant kingdom. Tannins are classified into two large groups: hydrolysable and condensed tannins (1) (Fig. 1A). Hydrolyzable tannins have structures in which a poly-alcohol (mainly glucose) is esterified with a polyphenolic carboxylic acid such as a galloyl, hexahydroxydiphenoyl (HHDP) (a dimer of the galloyl group), valoneoyl (a trimer of the galloyl group), or dehydrohexahydroxy-diphenoyl group (an oxidized metabolite of the HHDP group). Condensed tannins are composed of flavan units, mostly (+)-catechin, (−)-epicatechin, or their analogs, condensed with each other via carbon–carbon bonds.

*Corresponding author. sakagami@dent.meikai.ac.jp
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Invited article

Flavonoids are polyphenolic compounds that are secondary metabolites synthesized from chalcones and categorized into flavonols, flavones, flavanones, isoflavones, pterocarpan, coumestan, etc. (2) (Fig. 1B). Resveratrol, recently known for its anti-aging effect, is classified as a stilbenoid.

Lignins are formed through phenolic oxidative coupling processes. Lignin macromolecules are formed by the dehydrogenative polymerization of three mono-lignols: *p*-coumaryl, *p*-conifery, and sinapyl alchohols. Some polysaccharides in the cell walls of lignified plants are linked to lignin to form lignin-carbohydrate complexes (3) (Fig. 1C). The molecular weight, acidity, and solubility of the lignin-carbohydrate complex may be changed subtly, depending on the ratio of lignin and carbohydrate moieties. Due to the difficulty of complete structural determination, the pharmacological studies of lignin-carbohydrate complexes have been limited, as compared with those of tannins and flavonoids (4 – 7).

For the exploration and evaluation of new drugs,

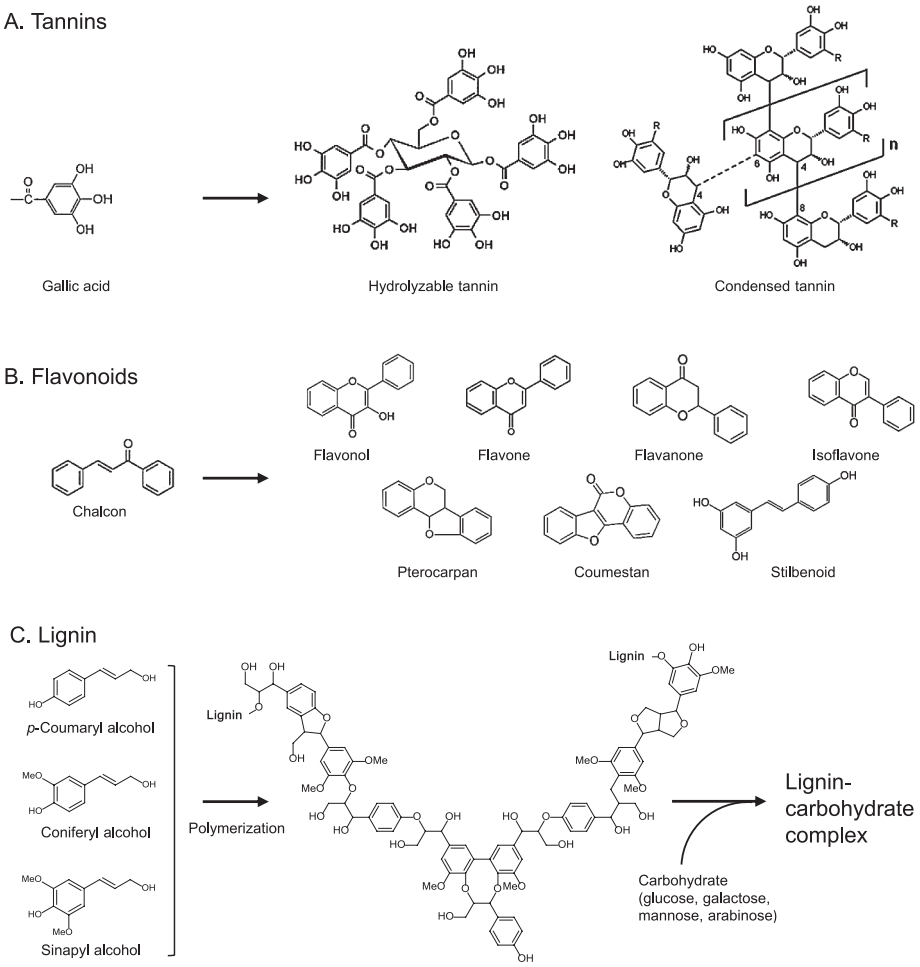

Fig. 1. Backbone structures of tannin (A), flavonoid (B), and lignin-carbohydrate complex (C). Pentagalloyl glucose is shown as an example for monomeric hydrolyzable tannins (A).

animal experiments are still mandatory. However, there are big differences in pharmacodynamics and pharmacokinetics of drugs between animals and humans. Some animal protection groups protest against animal experiments from the ethical point of view. These trends significantly reduce the support for the animal experiments. Recent progress in computer technology led researchers to explore metabolic profiling with a chemically designed liver and microtips that mimic the function of internal organs, thus minimizing the weight of animal experiments (8). The other approach is to use cultured cells that mimic the whole body. In order to investigate the effect of polyphenols on the oral diseases, we have established simple in vitro assay systems with human cultured cells (Fig. 2). The present article reviewed the biological activities and possible dental application of three major polyphenols, citing mostly our in vitro studies together with those from other groups.

2. Tumor-specific cytotoxicity against human oral squamous cell carcinoma

An in vitro assay system for tumor-specificity was established using human oral squamous cell carcinoma cell lines (HSC-2, HSC-3, HSC-4, Ca9-22) and human oral normal cells [gingival fibroblast (HGF), pulp cell (HPC), periodontal ligament fibroblast (HPLF)]. These cells were incubated for 48 h with increasing concentrations of test samples, and the relative viable cell number was determined by the MTT method. In brief, the treated cells were incubated for another 3 h in fresh culture medium containing 0.2 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). If cells are viable, the water-soluble MTT is reduced to an insoluble formazan having a purple color by NAD(P)H-dependent cellular oxidoreductase enzymes. The formazan is then solubilized with 0.1 ml of DMSO and the concentration (that reflects the relative viable cell

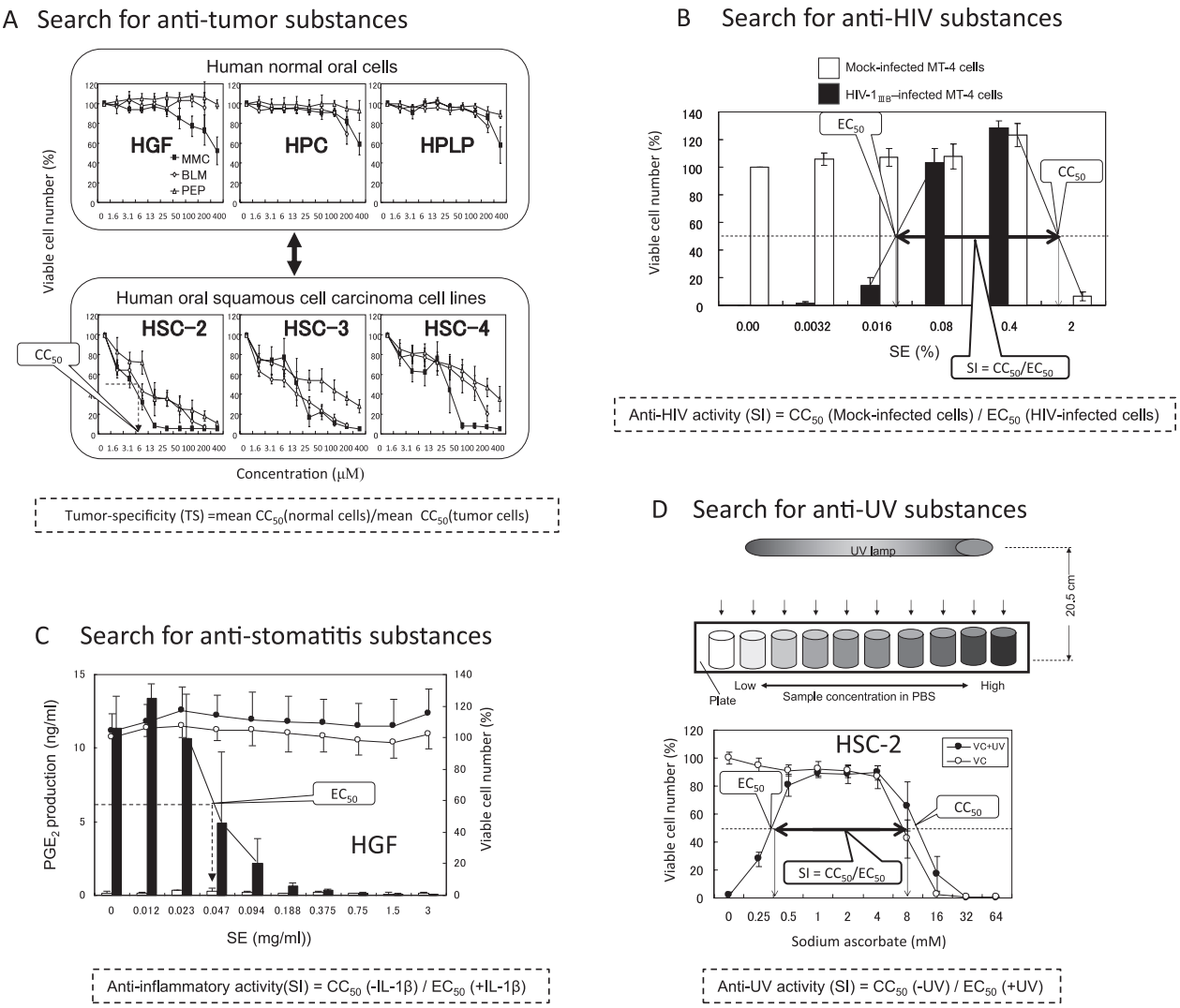

Fig. 2. In vitro assay systems used to evaluate the anti-tumor (A), anti-viral (B), anti-inflammatory (C), and anti-UV activity (D). Both human oral normal and tumor cells were treated for 48 h with the indicated concentrations of mitomycin (MMC), bleomycin (BLM), or peplomycin (PEP); and viable cell number was determined by the MTT method to calculate the tumor-specificity index (TS value) (A). Mock- or HIV-infected MT-4 cells were incubated for 5 days with different concentrations of alkaline extract of leaves of *Sasa senanensis* Rehder (SE), and the relative viable cell number was determined by MTT assay, to calculate the anti-HIV index (SI value) (B). Human gingival fibroblast (HGF) cells were incubated for 24 h with the indicated concentrations of SE in the presence (black symbols) or absence (white symbols) of IL-1*β* (5 ng/ml), and the viable cell number (circles) and extracellular PGE_2 concentrations (bars) were determined to calculate the anti-inflammatory index (SI value) (C). HSC-2 cells were exposed for 1 min to UV irradiation (closed circle) or not (open circle) in PBS(−) containing the indicated concentrations of sodium ascorbate and incubated for a further 48 h in fresh culture medium to determine the relative viable cell number and then calculate the anti-UV index (SI value). Each value represents mean ± S.D. of triplicate assays.

number) determined by optical density at 540 nm. Tumor-selectivity index (TS value) was determined by dividing the mean CC_{50} against normal cells by the mean CC_{50} against tumor cells (Fig. 2A).

Among 24 plant extracts, *Camptotheca acuminate* leaf showed the highest TS value (88.3), followed by *Vitis spp* (> 3.5), *Sasa veitchii* (> 2.3), and *Phellodendron amurense* (> 2.1), whereas other plant extracts showed much lower TS value (< 2) (9). Anticancer drugs such as camptothecin [originally isolated from the stem wood of the *Camptotheca acuminata* tree) (TS = 2961) (10), anthracyclines (doxorubicin, daunorubicin, epirubicin, mitoxantrone) (TS = 181 ± 100) (range: 47 – 259)] (11), mitomycin (TS > 23) (12), docetaxel (TS = > 128) (13), 5-FU (TS = > 66), and bacterial products (nocobactin NA-a, -b) (TS = 43.9 – 80) (14) showed higher tumor-

specificity (Table 1). Especially, topoisomerase I inhibitors [camptothecin (TS = 2961), SN-38, an active metabolite of irinotacan (TS = 1321)] and topoisomerase II inhibitors [etoposide (TS = 486), teniposide (TS = 3190)] showed the highest tumor-selectivity, suggesting future application for chemotherapy of oral squamous cell carcinoma (10). Although human normal oral cells (derived from mesenchymal tissues) and oral squamous cell carcinoma (derived from epithelial tissues) showed different cell types, the tumor-specificity determined by the present method seems to reflect the anti-cancer activity.

Lignin-carbohydrate complexes (TS = 2.7), flavonoids [flavonols, isoprenylflavonoids (TS = 2.1 ± 0.4) (15 – 17), 2-arylbenzofurans (TS = 1.2 ± 0.2) (18), benzophenones (TS = 1.7 ± 0.4), xanthones (TS = 1.2 ± 0.3) (19), anthraquinones (TS = 3.8 ± 4.9), phenylbutanone glycosides (TS = 1.5 – 3.3), stilbene glucosides (TS = 2.4 ± 0.8), naphthalene glucosides (TS = 1.1 – 1.4) (20), luteolin glucosides (21)] (TS = 1 – 3.8), tannins (TS = 1 – 4.8) (22, 23), terpenoids and their glycosides (TS = 1.1 – 2.5) (4, 24, 25) showed much lower tumor-specificity.

Many antioxidants expected for anti-aging effect, such as sodium ascorbate (vitamin C) (TS = 2.5), gallic acid (TS = 1.1), catechin (TS = 1.0), epigallocatechin gallate (TS = 4.1), chlorogenic acid (TS = 1.7) (26), daidzein (TS = 1.1), genistein (TS = 2.4) (27), quercetin (TS = 2.2), isoliquiritigenin (TS = 4.0), kaempferol (TS = 1.4), resveratrol (TS = 2.9) (28), and curcumin (TS = 1.7) (5) showed disappointingly lower tumor-selectivity (Table 1).

Among synthetic compounds, *α*,*β*-unsaturated ketones with ring structure such as 6-(4-nitrophenylmethylene)-2-(3,4,5-trimethoxyphenylmethylene)cyclohexanone (TS = > 176) (29), 2-{3-[3,5-bis(benzylidene)-4-oxopiperidin-1-yl]-3-oxopropylsulfanyl}ethanesulfonic acid (TS = 154) (30), 3,5-bis(benzylidene)-1-diethylphosphono-4-oxopiperidines (TS = 71) (31), 2-benzylidene-6-(nitrobenzylidene)cyclohexanones (TS = > 108) (32), and dimeric 3,5-bis(benzylidene)-4-piperidones (TS = 90) (33) showed higher tumor-selectivity. These compounds only slightly activated caspase-3, but did not induce internucleosomal DNA and fragmentation (biochemical hallmark of apoptosis) in human oral squamous cell carcinoma cell lines. Furthermore, codeinone (oxidative metabolite of codeine) and morphinone (oxidative metabolite of morphine), which have an *α*,*β*-unsaturated ketone structure did not induce apoptosis, but rather induced autophagy (formation of secondary lysosome) in HL-60 cells, which is known to be a very sensitive cell line to commit to apoptosis by many inducers (34). These results suggest that the apoptosis-inducing activity may not be necessarily related to the tumor-selectivity.

On the other hand, simple *α*,*β*-unsaturated carbonyl compounds (TS = 1.2 ± 0.3) (35), hydroxyketones (TS = 5.7 ± 6.0) (36), *β*-diketones (TS = 1.8 ± 1.4) (37), trifluoromethylketones (TS = 2.6 ± 1.6) (38), azulenequinones (TS = 2.6 ± 2.3) (39), vitamin K_2 derivatives (TS = 1.9 ± 0.2), and prenylalcohols (prenol, geraniol, farnesol, geranylgeraniol, geranylfarnesol) (TS = 1.3 ± 0.3) (40), coumarins (TS = 1.8 ± 0.9) (41), hydroxylated coumarins (TS = 2.4 ± 3.0) (42), azulene (TS = 1.7 ± 1.0) (43), trihaloacetylazulenes (TS = 6.5 ± 10.7) (44) and (TS = 1.7 ± 0.6) (45), water-soluble azulenes (TS = 2.3 ± 0.6) (46), tropolones (TS = 2.6 ± 1.8) (47), benzo[*b*]cyclohept[*e*][1,4]oxazine, and 2-aminotropones (TS = 2.3 ± 1.0) (48), benzocycloheptoxazines (TS = 3.6 ± 3.1) (49), 1,4-dehydropypyridine derivatives (1.6 ± 0.8) (50), 2-aminomethylene-3(2*H*)-benzofuranone (2.1 ± 1.7) (51), benzothiepins (TS = 2.4 ± 1.6) (52), 5-benzoylimidazoles (TS = 2.4 ± 1.1) (53), 4-trifluoromethylimidazoles (TS = 1.7 ± 1.0) (54), 3-formylchromones (TS = 2.5 ± 1.3) (55), phenoxazines (TS = 1.7 ± 1.1) (56), berberines (TS= 3.6 – 4.0) (57), sodium 5,6-benzylidene-L-ascorbate (SBA) (TS = 2) (58), benzaldehyde (TS = 8.8) (59), naphtha[2,3-*b*]furan-4,9-diones (TS = 3.5 ± 6.7) (0.3 – 36.9) (60), 2-styrylchromones (TS = 7.3 ± 6.1) (61), local anesthetics (lidocaine, mepivacaine, dibucaine, bupivacaine, procaine, tetracaine, aminobenzoate) (TS = 1.4 ± 0.42) (62) showed relatively lower tumor-specificity. Introduction of the dibenzyol group to dihydropyridines enhanced the tumor-specificity (TS = > 33 – > 53) (63).

Recently, we found that 3-styrylchrome derivatives showed moderate to high tumor selectivity. Especially, compounds that have a methoxy group at the 6-position of the chormone ring and a hydroxyl group at the 4′-position of the phenyl group in the styryl moiety showed the highest tumor-selectivity (TS = 69.0) (64). Multivariate statistics with chemical descriptors for the location of the substituted group, molecular shape, and electrostatic interaction may be useful for designing the most favorable compound with higher tumor selectivity.

3. Anti-viral activity

Virus infection is one of the risk factors, and therefore anti-viral substances are expected to reduce the incidence of carcinogenesis. In order to evaluate the anti-HIV activity in vitro, the lethal condition for human T-cell leukemia virus I (HTLV-I)-bearing CD4-positive human T-cell line MT-4 by HIV infection was established (multiplicity of infection = 0.01). Mock- and HIV-infected MT-4 cells (3×10^4 cells/96-microwell) were incubated for 5 days with different concentrations of samples and the relative viable cell number was deter-

Table 1. Tumor-specificity of polyphenols

Compounds (n: number of compounds)	Tumor-specificity (TS)	Ref.
Lignin-carbohyrate complexes derived from pine cones (n = 4)	2.7 ± 1.1 (1.7 – 4.1)	5, 7
Flavonoids		
Flavones, flavonols (n = 36)	1.2 ± 0.6 (0.3 – 3.2)	4
Flavonoids (n = 31)	3.2 ± 4.0 (0.8 – 31.7)	4
Isoprenylflavonoids (n = 22)	2.1 ± 0.4 (1.6 – 3.0)	4
2-Arylbenzofurans (n = 6)	1.2 ± 0.2 (1.0 – 1.5)	18
Benzophenones (n = 5)	1.7 ± 0.4 (1.2 – 2.3)	19
Xanthones (n = 9)	1.2 ± 0.3 (1 – 1.7)	19
Anthraquinones (n = 13)	3.8 ± 4.9 (1.0 – 18.6)	20
Phenylbutanone glycosides (n = 2)	2.4 (1.5 – 3.3)	20
Stilbene glucosides (n = 9)	2.4 ± 0.8 (1.0 – 4.7)	20
Naphthalene glucosides (n = 2)	1.3 (1.1 – 1.4)	20
Luteolin glucosides (n = 3)	1	21
Tricin, morin, quercetin, kaempferol	1.5 ± 0.6 (1 – 2.2)	28
Isoliquiritigenin, datiscetin, galangin	2.0 ± 1.7(1 – 4)	28
Resveratrol, daidzein, genistein	2.1 ± 0.9 (1.1 – 2.9)	27, 28
Tannin-related compounds		
Gallic acid, catachin	1.0 – 1.1	22
Epigallocatechin gallate (EGCG)	4.1	22
Procyanidins (n = 6)	4.8 ± 2.3 (1.0 – 7.4)	22
Hydrolyzable tannins (monomer) (n = 7)	1.5 ± 0.5 (1.0 – 2.5)	22
Hydrolyzable tannins (oligomers) (n = 3)	1.4 ± 0.2 (1.2 – 1.5)	22
Large circular ellagitannins (n = 4)	4.4 ± 2.7 (2.3 – 8.2)	23
Terpenoids and saponins		
Triterpenes (n = 18)	1.2 ± 0.7 (0.7 – 2.1)	4
Triterpene glycosides (n = 31)	1.2 ± 0.5 (1.0 – 1.8)	4
Cycloartane glycosides (n = 7)	1.1 ± 0.2 (0.9 – 1.4)	4
Furostaol glycosides (n = 17)	2.5 ± 4.1 (0.4 – 17.0)	4
Ketones		
α,β-Unsaturated ketones (n = 26)	1.2 ± 0.3 (0.6 – 1.9)	35
α,β-Unsaturated ketones with ring structure (n = 4)	> 229.0	29 – 33
α-Hydroxyketones (n = 8)	5.7 ± 6.0 (1.0 – 17.6)	36
β-Diketones (n = 22)	1.8 ± 1.4 (0.3 – 6.3)	37
Trifluoromethylketones (n = 6)	2.6 ± 1.6 (1 – 4.5)	38
Azulenequinones (n = 27)	2.6 ± 2.3 (1.0 – 10.2)	39
Other compounds		
Curcumin	1.7	5
Chlorogenic acid	1.7	26
Vitamin C	2.5	5
Vitamin K_2 derivatives (n = 3)	1.9 ± 0.2 (1.7 – 2.0)	40
Prenyl alcohols (n = 5)	1.3 ± 0.3 (1.0 – 1.8)	40
Coumarins (n = 21)	1.8 ± 0.9 (1.0 – 4.1)	41
Hydroxylated coumarins (n = 23)	2.4 ± 3.0 (1.0 – 11.0)	42
Azulenes (n = 27)	1.7 ± 1.0 (0.8 – 5.7)	43
Trihaloazulenes (n = 26)	6.5 ± 10.7 (1.3 – 44.1)	44
Trihaloazulenes (n = 20)	1.7 ± 0.6 (1.0 – 3.5)	45
Water-soluble azulenes (n = 8)	2.3 ± 0.6 (1.4 – 3.5)	46
Tropolones (n = 27)	2.6 ± 1.8 (1.0 – 9.9)	47
Benzo[*b*]cyclohept[*e*][1,4]oxazine and 2-aminotropones (n = 20)	2.3 ± 1.0 (1.2 – 4.4)	48
Benzocycloheptoxazines (n = 26)	3.6 ± 3.1 (0.8 – 12.5)	49
1,4-Dehydropypyridine derivatives (n = 41)	1.6 ± 0.8	50
3,5-Dibenzoyl-1,4-dihydropyridines (n = 2)	> 43.0 (> 33 – > 53)	63
2-Aminomethylene-3(2*H*)-benzofuranone	2.1 ± 1.7 (0.9 – 9.1)	51
Benzothiepins (n = 11)	2.4 ± 1.6 (0.6 – 5.4)	52
5-Benzoylimidazoles (n = 4)	2.4 ± 1.1 (0.9 – 3.5)	53
4-Trifluoromethylimidazoles (n = 14)	1.7 ± 1.0 (1.0 – 4.3)	54
3-Formylchromones (n = 16)	2.5 ± 1.3 (1.0 – 5.9)	55
Phenoxazines (n = 24)	1.7 ± 1.1 (1.0 – 4.8)	56
Berberines (n = 2)	3.8 (3.6 – 4.0)	57
Sodium 5,6-benzylidene-L-ascorbate (SBA)	2	58
Benzaldehyde	8.8	59
Naphtha[2,3-*b*]furan-4,9-diones (n = 36)	3.5 ± 6.7 (0.3 – 36.9)	60
2-Styrylchromones (n = 6)	7.3 ± 6.1 (1.1 – 17.4)	61
3-Styrylchromones (n = 15)	14.9 ± 18.8 (1.6 – 69.0)	64
Local anesthetics (n = 7)	1.4 ± 0.42 (1.1 – 2.2)	62
Nocobactins (bacterial products) (n = 2)	62.0 (43.9 – 80.0)	14
Anticanceer drugs		
Anthracyclines (n = 4)	181 ± 100 (47 – 259)	11
Mitomicin C	> 29	12
Bleomycin, peplomycin	> 3.8 ± 0.2	12
5-FU	> 56	64
Melphalan	11.1	13
Docetaxel	> 128	10
Camptothecin	2961	10
Camptotheca acuminate leaf extract	88.3	9
Poly-herbal formula	839	117

mined by MTT assay, to yield the CC_{50} and EC_{50} (that increased the viable cell number of the HIV-infected cells to 50%), respectively. The anti-HIV activity (selectivity index, SI) was determined by dividing the CC_{50} (mock-infected cells) by EC_{50} (HIV-infected cells) (Fig. 2B).

Alkaline extraction was found to be more effective than water extraction to obtain higher amounts of anti-HIV substances, regardless of the plant species. Water extracts of green tea leaves, oolong tea leaf, orange flow, and licorice root showed very weak anti-HIV activity; however, when the residue or fresh sample was extracted with alkaline solution, much higher anti-HIV activity was recovered (SI = 0.022 → 3, 0.033 → 13, 0.5 → 15, 4 → 42) (65, 66) (Table 2). This explains why lignin-carbohydrate complexes, prepared by alkaline extraction show prominent anti-HIV activity. We have previously reported lignin-carbohydrate complexes prepared from the cone extract of *Pinus parviflora* Sieb. et Zucc. (67), pine cone of *Pinus elliottii* var. Elliottii. (68), pine seed shell of *Pinus parviflora* Sieb. et Zucc. (69), bark of Catuaba casca (*Erythroxylum catuaba* Arr. Cam.) (70), cacao husk (71), and mass (72) from the beans of *Theobroma*, *Lentinus edodes* mycelia extract (L·E·M) (73), precipitating fiber of mulberry juice fractions (74), and the leaves of *Sasa senanensis* Rehder (75) showed higher anti-HIV activity (SI = 7 – 311). On the other hand, polysaccharides [except for sulfated polysaccharide (76)] (SI = 1), Kampo medicines (SI = 1), and its constituent plant extracts (SI = 1 – 4) (77), extracted by hot water, showed much lower anti-HIV activity. Among tannin-related compounds, only hydrolysable tannins showed some anti-HIV activity that was increased with oligomerization, in the order of monomer (SI = 1.8) < dimer (SI = 2.3) < trimer (SI = 3.4) < tetramer (SI = 7.3), whereas condensed tannins were inactive (SI = 1.1) (78). Flavonoids showed low anti-HIV activity (SI = 1.5 – 5.3) (15, 16, 18). Although luteolin glycosides (TS = 2 – 7) and tricin (SI = 24) showed slightly higher anti-HIV activity, but not to the extent attained by lignin-carbohydrate complexes (21) (Table 2).

Lignin-carbohydrate complexes inhibited the adsorption of HIV to target cells and reverse transcriptase activity (79, 80). Limited digestion experiments with chlorous acid (that degrades the lignin) and sulfuric acid (that degrades the carbohydrate) revealed that the lignin moiety, but not the carbohydrate moiety, is essential for anti-HIV activity expression (78) (Fig. 2). This point was confirmed by our finding that dehydrogenation polymers of phenylpropenoids (caffeic acid, ferulic acid, *p*-coumaric acid) synthesized in vitro that do not contain sugars showed slightly higher anti-HIV activity (SI = 50 – 100) (81). Since phenylpropenoid monomers were inactive (SI < 1) (81), polymerized structure of phenylpropenoids is essential for anti-HIV activity expression.

When radiolabeled lignin-carbohydrate complex was centrifuged on sucrose gradient centrifugation, it floated up on the top of the gradient. However, in the presence of influenza virus, it sedimented to the fraction that contained the virus, suggesting the tight binding of the lignin-carbohydrate complex with influenza virus (82). Lignin-carbohydrate complex inhibited the plaque formation and RNA polymerase activity in vitro (83, 84). Direct mixing of influenza virus with lignin-carbohydrate complex instantly diminished the infectivity of virus in mice (82). Similarly, dehydrogenation polymers of phenylpropenoids potently inhibited the plaque formation and RNA polymerase activity of influenza virus (85). Lignin-carbohydrate complex inhibited the plaque formation and adsorption of herpes simplex virus (HSV-1) to the cells (86) and cytophatic effects of rotavirus and enterovirus (87).

4. Anti-inflammatory activity in human gingivitis model

Oral inflammation such as stomatitis may be triggered or aggravated by many risk factors including bacterial and viral infections, nutritional deficiencies, declined immune functions, allergic reactions, radiotherapy, stress, cigarettes, and diseases. Topical steroids, matrix type transdermal patch, vitamins, throat lozenges, mouth wash, Kampo medicines, and cryotherapy are used for the treatment of stomatitis. However, there are cases in which such treatments are not effective, and therefore treatments with broader spectrum are desirable.

As an in vitro oral inflammation model, IL-1β-stimulated human gingival fibroblasts (HGF) were used. When HGF cells were stimulated with IL-1β (5 ng/ml), one or two orders higher concentrations of inflammatory cytokines (IL-6, IL-8, MCP-1) and PGE_2, but not TNF-α and nitric oxide (NO), were produced and released into the culture medium (88). The selective index (SI) for the anti-inflammatory activity was determined by dividing the CC_{50} (against unstimulated HGF cells) by the EC_{50} (concentration that inhibits the PGE_2 production by 50% in IL-1β-stimulated HGF cells). Alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) inhibited the PGE_2 production (SI = > 75.8) in this oral inflammation model (Fig. 2C) (89) more effecively than rikkosan (Lot No. 2990110010), a Kampo medicine clinically used for the treatment of stomatitis (SI = > 4.0) (90). SE also potently inhibted the production of IL-8 by IL-1β-stimulated HGF cells (88) and PGE_2 production by IL-1β-stimulated human periodonal ligament fibroblasts

Table 2. Anti-HIV activity of polyphenols

Samples (n = number of samples or compounds tested)		Anti-HIV activity (SI)	Ref.
Green tea leaves	Hot water extraction	< 0.022	65
	Hot water extraction → Alkaline extraction	< 0.11	
	Alkaline extraction	3	
Oolong tea leaves	Hot waer extraction	< 0.033	65
	Hot water extraction → Alkaline extraction	9	
	Alkaline extraction	13	
Orange flower	Hot water extraction	< 0.5	65
	Hot water extraction → Alkaline extraction	13	
	Alkaline extraction	> 15	
Licorice root	Hot water extraction	4	66
	Alkaline extraction	42	
Lignin-carbohydrate complex			
Pine cone of *Pinus parviflora* Sieb. et Zucc.		14	67
Pine cone of *Pinus elliottii var. Elliottii*		28	68
Pine seed shell of *Pinus parviflora* Sieb. et Zucc.		12	69
Bark of *Erythroxylum catuaba* Arr. Cam.		43	70
Husk of cacao beans of *Theobroma*		311	71
Mass of cacao beans of *Theobroma*		46	72
Lentinus edodes mycelia extract (L·E·M)		94	73
Precipitating fiber fraction of murberry juice		7	74
Alkaline extract of leaves of *Sasa senanensis* Rehder		86	75
Dehydrogenation polymers of phenylpropenoids (n = 23)		105	81
Polysaccharides			
Neutral polysaccharides of pine cone of *P. parviflora* Sieb. et Zucc.		1	7
Uronic acid-containing polysaccharides of pine cone		1	7
N,N-Dimethylaminoethyl paramylon (substituion ratio: 5%)		< 1	76
N,N-Diethylaminoethyl paramylon (substitution ratio: 10%)		< 1	76
2-Hydroxy-3-trimethylammoniopropyl paramylon chloride		< 1	76
Sodium paramylon sulfate (substitution ratio: 4%)		> 274	76
Dimethylaminoethyl curdlan (substitution ratio: 5%)		< 1	76
PSK (protein-bound polysaccharide)		1	76
Kampo medicines (n = 10)		1.0 ± 0.0	77
Constituent plant extracts of Kampo medicines (n = 25)		1.3 ± 0.8 (1 – 4)	77
Tannin-related compounds			
Hydrolyzable tannins (monomer) (MW: 484-1255) (n = 21)		1.8 ± 2.8 (1 – 13)	78
Hydrolyzable tannins (dimer) (MW: 1571-2282) (n = 39)		2.3 ± 3.2 (1 – 15)	78
Hydrolyzable tannins (trimer) (MW: 2354-2658) (n = 4)		3.4 ± 3.7 (1 – 10)	78
Hydrolyzable tannins (tetramer) (MW: 3138-3745) (n = 3)		7.3 ± 6.5 (1 – 14)	78
Condensed tannins (MW: 290-1764) (n = 8)		1.1 ± 0.4 (1 – 2)	78
Flavonoids			
Flavonoids (n = 92)		1.5 ± 1. 9 (1 – 12)	18
Prenylated isoflavones (n = 10)		1.8 ± 1.4 (1 = 5)	15
Isoflavones (n = 8)		< 1	16
Luteolin glucosides (MW: 419-449) (n = 3)		5.3 ± 2.9 (2 – 7)	21
Tricin (MW: 331)		24	21
Anti-HIV drugs			
Dextran sulfate (molecular mass, 5 kDa)		2956	
Curdlan sulfate (molecular mass, 79 kDa)		11718	
Azidothymidine		23261	
2′,3′-Dideoxycytidine (ddC)		2974	

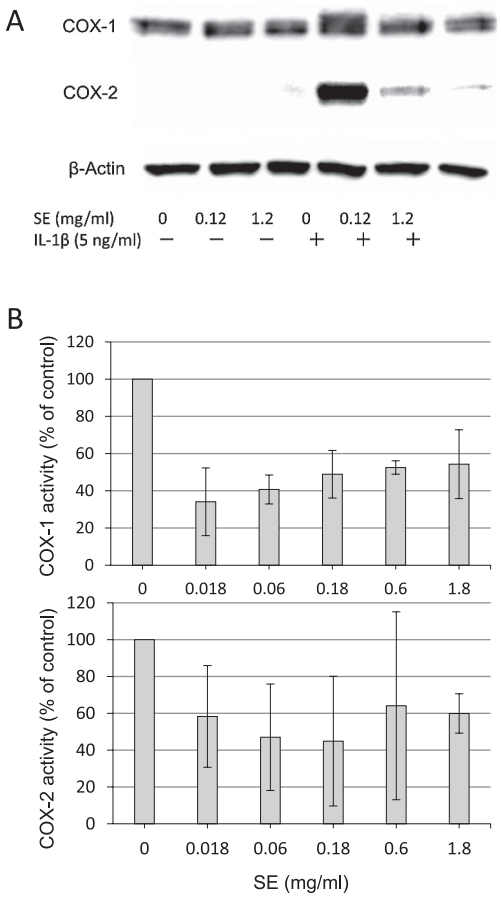

Fig. 3. Effect of alkaline extract of leaves of *Sasa senanensis* Rehder (SE) on COX-1 and COX-2 protein expression (assessed by western blot analysis) (A) and enzyme activity (B). Enzyme activity was determined by the amount of PGF2α produced in the enzyme reaction, according to Cox Inhibitor Screening Assay Kit (Cayman Company). Each value represents the mean ± S.D. from 3 independent experiments.

(SI = 96.8) (89). Possible mechanisms of this anti-inflammatory action of SE are mediated by preferential inhibition of COX-2 protein expression (A) and partial inhibition of both COX-1 and COX-2 enzyme activity (B) (Fig. 3) (Sakagami et al., abstract, The 56th Annual Meeting of Japanese Association for Oral Biology, September 2014). Metoboromic analysis is underway to identify the intracellular target molecules. It remains to be determined whether lignin-carbohydrate complexes and other polyphenols show similar anti-inflammatory action in this system.

Kampo medicines, Shosaikoto (TJ-9) (91) and Orento (TJ-120) (92) inhibited the *Porphyromonas gingivalis* LPS–induced PGE_2 production, but not that of IL-6 and IL-8 production in human gingival fibroblasts. On the other hand, Rokumigan (TJ-87) inhibited the secretion of IL-6 but not IL-8 by *Fusobacterium nucleatum*–stimulated epithelial cells and gingival fibroblasts (93). Curcumin inhibited the COX-2 mRNA and protein synthesis in *P. gingivalis* LPS–stimulated human gingival fibroblasts possibly due to the inhibition of the NF-κB pathway (94). Curcumin may also induce anti-inflammatory action via inhibition of Ca^{2+}-release–activated Ca^{2+} channels and K^+ channels in lymphocytes when it is orally administered (95). However, considering the limited pharmacokinetic availability of orally taken curcumin, careful interpretation is required for determining the effects in vivo (95). Recent studies suggest the possibility that α,β-unsaturated carbonyl based compounds might serve as the leading molecules for the design and development of improved anti-inflammatory agents (96).

5. Anti-UV activity

Ultraviolet rays (UV) are invisible electromagnetic waves. Moderate doses of UV exert several favorable effects, such as sterilization and disinfection, induction of vitamin D synthesis, and stimulation of metabolism and skin resistance, whereas an excessive dose of UV produces reactive oxygen species (ROS) that damage cellular DNA and proteins, leading to carcinogenesis. Molecular alterations in cutaneous neoplasms of the head and neck are often related to UV exposure (97).

We recently established a method for measuring the activity to protect the cells from UV-induced injury (referred to as 'anti-UV activity'), using UV-sensitive human oral squamous cell carcinoma HSC-2 cells (Fig. 2D). HSC-2 cells were replenished with phosphate-buffered saline with calcium and magnesium [PBS(−)] containing different concentrations of samples. The cells were then placed at 20.5 cm from a UV lamp (wavelength = 253.7 nm), taking off the lid, and exposed to UV irradiation (6 J/m^2 per min) for 1 min. The media were immediately replaced with fresh culture medium and cells were cultured for a further 48 h to determine the relative viable cell number by MTT method. From the dose–response curve, the CC_{50} against unirradiated cells and the concentration that increased the viability of UV-irradiated cells to 50% (EC_{50}) were determined. The selectivity index (SI) was determined by the following equation: $SI = CC_{50}/EC_{50}$. UV irradiation, followed by 48-h incubation resulted in almost complete loss of viable cells due to non-apoptotic cell death characterized by the lack of internucleosomal DNA fragmentation. However, addition of sodium ascorbate (vitamin C) at the time of UV irradiation potently inhibited the UV-induced cell death (Fig. 1D). Since anti-oxidant *N*-acetyl-L-cysteine and catalase could not prevent the UV-induced cell death, hydrogen may not be involved in UV-induced cytotoxicity (Table 3).

Table 3. Anti-UV activity of polyphenols

Samples (n = nubmer of samples or compounds tested)	Anti-UV activity (SI)	Ref.
Lignin-carbohydrate complex		98
Pine cone extract (n = 3)	33.4 ± 7.4 (24.8 – 38.1)	
Pine seed shell extract	25.6	
Lentinus edodes mycelia extract (L·E·M)	41.9	
Leaves of *Sasa senanensis* Rehder	38.5	
Vanillin	63.8	
Kampo medicines (n = 10)	2.4 ± 1.8 (1 – 4.9)	77
Constituent plant extracts of Kampo medicines (n = 25)	1.4 ± 1.6 (1 – 8)	77
Tea extracts		
Green tea leaves	3.4	99
Black tea leaves	< 1	
Jasmine tea leaves	< 1	
Coffee	9.6	
Pet-bottle of tea extracts		99
Oolong tea leaves	< 1	
Green tea leaves	1.6	
Oh-ki tea leaves	< 1	
Burley tea leaves	< 1	
Turmeric extract	< 1	99
Curcumin	< 1	
Ar-turmerone	< 1	
Antioxidants		
Sodium ascorbate (vitamin C)	42.4	99
Gallic acid	5.4	
Epigallocatechin gallate	7.7	
N-Acetyl-L-cysteine	< 1	
Catalase	< 1	
Water-soluble azulenes (n = 8)	35.5 ± 21.6 (4.5 – 65.6)	46

Lignin-carbohydrate complexes (SI = 24.8 – 38.1) (98), *Lentinus edodes* mycelia extract (LEM) (SI = 41.9) (99), and SE (SI = 38.5) (100) showed comparable anti-UV activity with vitamin C. On the other hand, Kampo medicine (SI = 1 – 4.9), its constituent herb extracts (SI = 1 – 8) (77), green tea, black tea, jasmine tea, ohki-cha, and burley tea leaf extracts (SI = 1 – 3.6) (99) showed much lower anti-UV activity.

Among synthetic compounds, newly synthesized water-soluble azulenes showed potent anti-UV activity (46). These anti-UV samples are promising for application to skin care products.

6. Anti-bacterial activity

The oral cavity contains almost half of the approximately 700 species of commensal microorganisms that are present in the human body. Hinokitiol, an aromatic seven-membered tropolon and a component of essential oils isolated from *Cuoressaceae*, inhibited the growth of *Candida albicans*, an opportunistic pathogen causing serious local and systemic infections especially in elderly and HIV-positive patients (101). A short-time treatment (30 min) with hinokitiol inhibited the adherence of *C. albicans* to oral epithelial cells and biofilm formation, but did not inhibit the growth of *C. albicans*. On the other hand, long-time treatment and a high concentration of hinokitiol inhibited the adherence of *C. albicans*, and damaged both commensal bacterial and epithelial cells (102). Gel-entrapped cathechin inhibited the growth of the *Actinomyces* and *C. albicans*, but did not inhibit the growth of the oral streptococci that are important in the normal oral flora (103).

7. Interaction with vitamin C

Vitamin C shows two distinct actions, reducing and oxidizing actions, depending on the experimental condi-

tions. In the presence of water and oxygen, vitamin C produces hydrogen oxide and injures the cells (104, 105). There was a positive correlation between the radical intensity and cytotoxicity in vitamin C derivatives (sodium L-ascorbate, L-ascorbic acid, D-isoascorbic acid, 6-*β*-D-galactosyl-L-ascorbate, sodium 5,6-benzylidene-L-ascorbate) (106). Lignin-carbohydrate complex enhanced both the radical intensity and cytotoxicity of vitamin C, whereas epigallocatechin gallate, gallic acid, and tannic acid inhibited were inhibitory (104). Lignin-carbohydrate complex also enhanced the superoxide and hydroxyl radical scavenging activity (71, 74, 107), anti-UV activity (99, 100), and oxygen consumption (hypoxia-inducing activity) (108) of vitamin C.

8. Signaling pathway

Lignin-carbohydrate complex is composed of two major components: lignin and carbohydrate. Limited digestion of lignin-carbohydrate complex suggests that the lignin moiety is involved in the prominent anti-HIV activity, whereas the carbohydrate moiety is involved in immunopotentiating activity (6, 7) (Fig. 4). Using DNA microarray analysis, we have recently reported that treatment of mouse macrophage-like J774.1 cells with lignin-carbohydrate complex isolated from LEM (Fr4) enhanced the expression of dectin-2 (4.2-fold) and 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); Sykb; Zap70; Jak2 (1.0- to 1.2-fold); Nf*κ*b1; NF*κ*b2; Rela, Relb (1.0- to 1.6-fold); Nf*κ*bia, Nf*κ*bib, Nf*κ*bie, Nf*κ*bi12 Nf*κ*biz (0.8- to 2.3-fold). On the other hand, LPS did not affect the expression of dectin-2 nor TLR-2 (109). These data suggest the significant role of the activation of the dectin-2 signaling pathway in the action of lignin-carbohydrate complex on macrophages (Fig. 2). It is generally accepted that dectin-2 is the receptor for mannan, whereas dectin-1 is that for glucose (110 – 112). It remains to be investigated whether dectin-2 is responsible for the immunopotentiation activity of lignin-carbohydrate complex against oral cells such as gingival fibroblasts, by performing the knock-down experiments with siRNA for dectin-2.

9. Clinical application

Oral uptake of lignin-ascorbic acid combination tablet significantly improved the symptoms of HSV-infected patients (113). Potent antiviral, antibacterial, and anti-inflammatory activity of alkaline extracts of leaves of *Sasa senanensis* Rehder (SE) prompted us to investigate whether SE is effective on oral lichenoid dysplasia and osteoclastogenesis. A male patient with white lacy streaks in the oral mucosa was orally administered SE three times a day for ten months. Long-term treatment cycle of SE progressively reduced both the area of white steaks and the base-line levels of salivary IL-6 and 8. IL-8 concentration after SE treatment was below the initial level throughout the experimental period. This

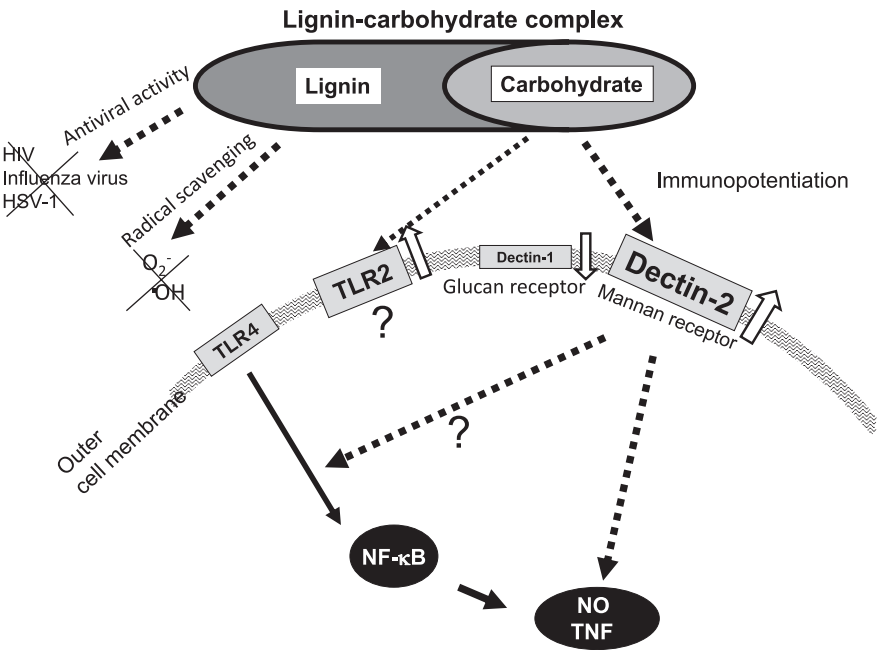

Fig. 4. Putative mode of action of lignin-carbohydrate complex.

was accompanied by the improvement of the patient's symptoms. SE significantly inhibited the RANKL-induced differentiation of mouse macrophage-like RAW264.7 cells towards osteoclasts (evaluated by TRAP-positive multinuclear cell formation). These pilot clinical studies suggest the therapeutic potentiality of SE against oral diseases (114). Tea polyphenols such as epigallocatechin-3-gallate and theaflavin-3,3′-digallate also inhibited the osteoclast formation and differentiation in vitro (115).

Clinical application of gel-entrapped hinokitiol inhibited the attachment of *C.abicans* to the tongue and the inflammatory cytokine production (IL-1β, IL-1α) (116).

10. Summary and future directions

This review provides the data base for assessing the possibility of application of three representative polyphenols to oral diseases. Most of the three major polyphenols (lignin-carbohydrate complex, tannins, flavonoids) showed much lower tumor-specificity against oral squamous cell carcinoma, in comparison to chemotherapeutic drugs. These disappointing observations suggest that the utilization of polyphenols for the therapy of oral cancer is not recommended, despite the numerous publications about the apoptosis induction by lower molecular weight polyphenols. Since the tumor-specificity and apoptosis-inducing activity are not necessarily overlapped with each other, the tumor-specificity of each candidate compound should be confirmed before the extensive research aiming at their clinical application. However, it is also important for us to continue or challenge to find a new antitumor substance using the present screening system since we have previously reported that some poly-herbal formulas showed extensively higher tumor-selectivity (TS = 839) (117). Potent anti-UV activity of lignin-carbohydrate complex may lower the incidence of head and neck cancer generated by UV exposure (97).

Lignin-carbohydrate complex, which can be obtained by alkaline extraction at higher yield than by water extraction, showed the highest anti-HIV activity. Also alkaline extracts of various plant materials showed much higher anti-HIV activity as compared with their water extracts. We thus recommend the use of alkaline solution rather than hot water to obtain higher yield of antiviral substances from plants. However, it remains to be investigated whether alkaline treatment may cause the degradation, molecular association with other components such as chlorophyll (7), and LPS contamination (118). We have previously reported that only 1.3% – 1.6% of orally administered lignin-carbohydrate complex appeared in the blood (119). Considering the low absorption through the intestinal tract, the application through the oral mucosa is recommended.

Oral care shortens the latent time of swallowing reflex presumably due to stimulating the nociception of the oral cavity. A combination of these sensory stimuli by foods with menthol or any other natural product candidates may improve swallowing disorders and prevent aspiration pneumonia (120). Since the administration of Kampo formulation ameliorated the cognitive function and emotional/psychiatric symptom-related behavior in animals (121), clinical evaluation of these formulae for dementia therapy is crucial.

In contrast to studies of inflammation and carcinogenesis, the studies of inflammation and aging were investigated much less. It has recently been reported that the inhibition of inflammation may prolong longevity (122). The anti-aging effects of antioxidants and anti-inflammatory substances including naturally distributing polyphenols remain to be investigated. To do this, it is essential to establish the evaluation systems with appropriate aging markers.

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Conflicts of Interest

The author declares no conflicts of interest.

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