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RESEARCH NOTE

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β-Glucuronidase Inhibitory Activity of Bromophenols Purified from *Grateloupia elliptica*

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Abstract β-Glucuronidases of intestinal bacteria are capable of retoxifying compounds that have been detoxified by liver glucuronidation, which is one of the most important detoxication processes in the liver. Therefore, this enzyme is known to accelerate colon cancer invasion and metastasis. Two bromophenols, 2,4,6-tribromophenol (I) and 2,4-dibromophenol (II), were purified from the red alga *Grateloupia elliptica*. IC_{50} values of bromophenol I and II against *Escherichia coli* β-glucuronidase were 5.4 and 8.5 mg/mL, respectively. Hence, bromophenols of *G. elliptica*, a potent β-glucuronidase inhibitor, can be used as a novel pharmaceutical agent for the prevention and treatment of colon cancer.

Keywords: β-glucuronidase, bromophenol, inhibitor, red algae, colon cancer

Introduction

The bacterial inhabitants of the human gastrointestinal tract constitute an enormously complex ecosystem that includes both aerobic and anaerobic microorganisms. There are more than 400 bacterial species in the colonic flora of a single individual (1). Epidemiologic studies have established that environmental factors influence the incidence of colon cancer. The disease is more frequent in North Americans and Western Europeans than residents in Africa, Asia, and South America (2). The critical difference between these groups appears to be the characteristic western diet, which is high in beef, fat, and protein. There was direct correlation between per capita beef consumption and the incidence of colon cancer (2). The colonic microflora is a metabolically active soup that produces enzymes that mediate these reactions with the production of mutagenic substances (3). These enzymes include β-glucuronidase, β-glucosidase, β-galactosidase, nitroreductase, azoreductase, 7-α-dehydroxylase, and cholesterol dehydrogenase (4). β-Glucuronidase (E.C.3.2.1.31) is widely distributed in animals, plants, and bacteria (5). Gastrointestinal bacteria known to produce this enzyme are *clostridia*, *bacteroidacea*, *eubacteria*, *peptostreptococci*, and *bifidobacteria*. *Clostridia* showed the highest β-glucuronidase activity; *bacteroidacea*, *eubactria,* and *peptostreptococci* showed less; *bifidobacteria* showed almost no activity (6). This enzyme is capable of retoxifying compounds that have been detoxified by liver glucuronidation, which is one of the most important detoxication processes in the liver. A number of carcinogens, including *N*-hydroxy-*N*-2-fluorenylacetamide and diethylstilbesterol, are activated by hydrolysis of the glucuronide bound in the bowel (7,8). Xenobiotics are molecules that are foreign to a living organism, and during the process of detoxification, they are conjugated with UDP-D-glucuronic acid in the liver. The resulting β-D-glucuronides are excreted through the small intestine and kidney. However, β-glucuronidase in the small intestine is known to hydrolyze the conjugates, and the freed xenobiotics are absorbed through the wall of small intestine and returned to the liver. β-Glucuronides are final metabolites of hydrophobic xenobiotics. Because β-glucuronides are excreted via urine and feces, they play important roles in the excretion of xenobiotics (9). However, β-glucuronidase produced by intestinal bacteria hydrolyzes glucuronide to liberate xenobictics, which exhibit toxicity in the intestine and decrease excretion rate of xenobiotics via reabsorption. Inhibition of bacterial β-glucuronidase in the intestine will promote excretion of xenobiotics and thus decrease their toxicity (10). Thus, it would be of considerable importance to develop β-glucuronidase inhibitor.

β-Glucuronidase inhibitors are mainly purified from terrestrial plants; *Scoparia dulcis* (11,12), *Ganoderma lucidum* (13), *Paeonia emodi* (14), *Baphia racemosa* (15), *Glycyrrhiza uralensis* (16), *Galla rhois* (17), root vegetables (18), *Lentinus edodes* (19), *Scutellaria viscidula* Bge (20), *Zizyphi fructus* (21), *kampo* (Japanese herb) medicine (22), and from purine nucleotides of dried bonito (23).

Seaweeds are rich in polysaccharides with high dietary content and known to contain various carbohydrase inhibitors. Most researches focused on α-glucosidase (24-26), β-D-glucanase (27), and aldose reductase inhibitors (28). On the other hand, Sekikawa *et al.* (9) reported that methanol extracts of *Chondria crassicaulis* inhibited β-glucuronidase but could not purify the inhibitory component. They found that the inhibitory components differed from each other because of the different inhibition mechanisms.

The objectives of this study were to screen and purify β-glucuronidase inhibitors from seaweeds collected in the eastern coastal area of Korean peninsula and to determine the structure and inhibitory activity of β-glucuronidase inhibitors of red algae *Grateloupia elliptica*.

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Materials and Methods

Reagents β-Glucuronidase and *p*-nitrophenyl-β-D-glucuronide (pNPG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sephadex LH-20 and Sephacryl HR-100 were purchased from Pharmacia Biotech Ltd. (Uppsala, Sweden). The other chemicals used in this study were of analytical grade.

Algal samples Two green algae (*Ulva pertus* and *Ostria costata*), 4 brown algae (*Sagassum horneri, Sagassum thunbergii, Sagassum nigrifolium*, and *Myelophycus simplex*), and 4 red algae (*G. elliptica, Plocaminum telfriae, Grateloupia lanceolata*, and *Polyopes lancifolia*) were harvested in the eastern coastal area of Korean peninsula in July and August, 2005. Fresh algae were washed with tap water and air-dried in the shade at room temperature. Dried sample was cut into small pieces (2×3 cm), homogenized, sifted with a 500-μm sieve, and then stored at –40°C until used.

Extraction and purification of β-glucuronidase inhibitors β-Glucuronidase inhibitor was purified according to the modified method of Kim *et al.* (29). The alga sample (1 kg) was soaked in 1 L of methanol-H_2O (80:20, v/v) and extracted with reflux for 3 hr. The solvent extract of the alga was filtered and evaporated to 200 mL under reduced pressure at <40°C. The evaporated was mixed with same amount of distilled water and then partitioned using *n*-hexane:H_2O (1:1). The aqueous layer was further partitioned using $CHCl_3$, ethyl acetate, and butanol. The EtOAc extract with highest β-glucuronidase inhibitory activity was loaded onto a silica gel column (2.0×15.0 cm) and then subjected to gradient elution by increasing polarity with mixtures (5:1, 4:1, 3:1, 2:1, and 1:1, v/v) of $CHCl_3$-MeOH. The fractions eluted with mixtures (3:1 and 5:1, v/v) of $CHCl_3$-MeOH were further purified by Sephadex LH-20 (3.0×30.0 cm) chromatography with methanol as an eluent. Extractive yield of inhibitor was determined by weighting after removing solvent with a vacuum evaporator (RE121 Rotavapor; Buchi, Flawil, Switzerland).

Inhibitory assay of β-glucuronidase activity β-Glucuronidase inhibitory activity was determined according to the modified method of Kurihara *et al.* (24) and Jun *et al.* (30). A 0.01 mL of 10 mM pNPG as a substrate and 0.01 mL of 20 U/mL β-glucuronidase in 0.01 M phosphate buffer (pH 7) were added to 2.2 mL sample solution to start the reaction. The reaction was carried out at 37°C for 60 min and stopped by adding 1.5 mL of 0.1 M Na_2CO_3. Enzymatic activity was quantified by measuring the absorbance at 405 nm. One unit of β-glucuronidase activity was defined as the amount of enzyme liberating 1.0 mM of *p*-nitrophenol/min. One unit of β-glucuronidase inhibitory activity was defined as one unit decrease of β-glucuronidase activity.

Identification of β-glucuronidase inhibitor The structures of β-glucuronidase inhibitors were identified by spectroscopic methods including ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and electron impact-mass spectrometry (EI-MS) (31). ^{1}H and ^{13}C-NMR spectra were recorded in methanol-d_1 (MeOD) on a Bruker DRX-600 spectrometer (Bruker, Karlsruhe, Germany). Mass spectra were recorded on JMS-AX500 and JMS-SX102A spectrometers (Jeol, Tokyo, Japan).

2,4,6-Tribromophenol (I) ^{1}H NMR (600 MHz, MeOD) δH 7.61 (2H. *s*. H-3); ^{13}C NMR (150 MHz, MeOD) δc 152.1 (C-1), 112.6 (C-2,6), 135.4 (C-3,5), 112.9 (C-4).

2,4-Dibromophenol (II) ^{1}H NMR (600 MHz, MeOD) δH δ 7.58 (1H, *d*, J=2.4 Hz, H-3), δ 7.28 (1H, *dd*, J=2.4, 9.0 Hz, H-5), δ 6.81 (1H, *d*, J=9.0 Hz, H-6); ^{13}C NMR (150 MHz, MeOD) δc 155.1 (C-1), 111.7 (C-2). 136.0 (C-3), 112.0 (C-4), 132.4 (C-5), 118.6 (C-6).

Statistical analysis Statistical analysis of the data was carried out by Duncan's multiple comparison test ($p \leq 0.05$) using the SPSS software package version 10.0 program from SPSS Inc. (Chicago, IL, USA).

Results and Discussion

Extraction and isolation of β-glucuronidase inhibitor The inhibitory activity of aqueous methanol extract of *G. elliptica* (30.2%) at 5 mg/mL was highest followed by *P. lancifolia* (28.40%), *S. nigrifoliumi* (25.2%), and *G.lanceolata* (23.2%) in order (data not shown). Red algae showed relatively high β-glucuronidase inhibitory activity. Therefore, *G. elliptica* was selected as a raw material for the purification of β-glucuronidase inhibitor in this study. Eight % methanol was the best extractive solvent followed by 80% acetone and 100% methanol in order (data not shown). Therefore, 80% methanol was used to extract β-glucuronidase inhibitor from *P. elliptica* in this study. Methanol extracts of the red alga, *C. crassicaulis*, inhibited *E. coli* β-glucuronidase (9), which was same as in this study.

Pigment including flavonoids and lipid components are contained in the extracts of methanol and acetone. Flavonoids in plants are known to function as shields against ultraviolet (UV) light, as attractants for pollination and oviposition, as signals for *N*-fixing bacteria, as antimicrobial/antiviral agents, and as plant defensive compounds (phytoalexins) (32). In the consecutive solvent fractionations of inhibitors, β-glucuronidase inhibitory activities of EtOAc fraction at the concentration of 0.1, 1.0, and 4.0 mg/mL were 12, 35, and 51%, respectively, whereas those of butanol fractions were 8, 13, and 15% (Table 1). However, there were no β-glucuronidase inhibitory activities in *n*-hexane, $CHCl_3$, and water fractions. Lipids, lead, chlorophyll, refined oil, and sterol, etc are dissolved and fractionated in *n*-hexane, and resin is usually extracted in $CHCl_3$. In addition, polyphenols such as flavonoid and tannin are extracted in EtOAc, and water-soluble components are shifted over in BuOH (33). A large amount of solute in 80% methanol extract was shifted over to *n*-hexane (52%) and $CHCl_3$ (26%), but the bulk is not β-glucuronidase inhibitors (Table 1). Based on above results, β-glucuronidase inhibitor of *G. elliptica* is considered to be a polar group like polyphenols.

Table 1. β-Glucuronidase inhibitory activities of the solvent-partitioned fractions of *Grateloupia elliptica* at different concentrations

Fractions	Yield (%) of 80% methanol extract	β-Glucuronidase inhibitory activity (%)		
		0.1 mg/mL[1)]	1.0 mg/mL	4.0 mg/mL
n-Hexane	52	NI	NI	NI
Chloroform	26	NI	NI	NI
Ethyl acetate	16	12[a]	35[a]	51[a]
Butanol	5	8[b]	13[b]	15[b]
Water	1	NI	NI	NI

[1)]The final concentration in the reaction mixture; NI, no inhibition; [a,b]means in the same column with different superscripts are significantly different ($p<0.05$).

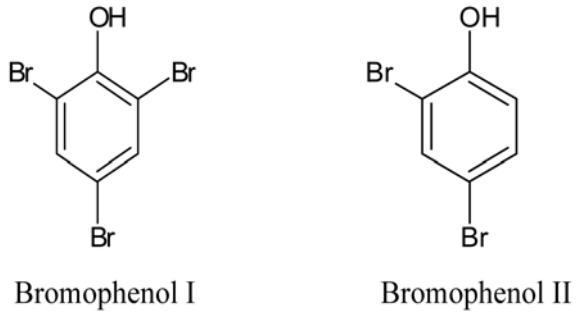

Fig. 1. The structures of β-glucuronidase inhibitors purified from *Grateloupia elliptica*.

Purification and identification of β-glucuronidase inhibitor EtOAc fraction was further purified by silica gel chromatography, which was eluted with $CHCl_3$-MeOH gradient. The highest β-glucuronidase inhibitory activity and extraction yield were obtained in the fraction of $CHCl_3$:MeOH=70:30 eluent (data not shown). Each fraction of $CHCl_3$-MeOH was loaded on the plate of silica gel thin layer chromatography (TLC) and then developed with the solvent of toluene:EtOAc:acetic acid=5:7:1. The plate was sprayed with $FeCl_3$ solution to analyze spot pattern, where positive reaction of blue color was confirmed. Therefore, β-glucuronidase inhibitor was estimated to be a kind of phenol compound (25). β-Glucuronidase inhibitor fraction of $CHCl_3$:MeOH=70:30 was concentrated by vacuum-evaporator and then loaded onto Sephadex LH-20 chromatography which was eluted with methanol, where total 30 fractions were obtained. Two kinds of positive spots on the plate of TLC among 30 fractions were confirmed and then classified into compound I (30.1 mg) and compound II (22.1 mg). Sephadex LH-20 gel (exclusion limit about 5,000 Da) used in this study was known to be very effective to remove high molecular compounds and polymers (34). The structures of β-glucuronidase inhibitors from *G. elliptica* were identified as bromophenol I and bromophenol II according to spectral data (Fig. 1), which was similar to the results of Kurihara *et al.* (25), where 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol and 2,3-dibromo-4,5-dihydroxybenzyl alcohol were purified from *Symphyocladia latiuscula* and *Odonthalia corymbifera*, respectively. β-Glucuronidase inhibitors are categorized as terpenoids and their glucuronides, flavonoids, and their glucuronides, and pseudo-sugar containing nitrogen (9), in which bromophenols are classified into flavonoids.

Table 2. IC_{50} values of *Grateloupia elliptica* bromophenols against β-glucuronidase[1)]

Inhibitor	IC_{50}[2)] (mg/mL)
Bromophenol I	5.4[b]
Bromophenol II	8.5[a]

[1)]Enzyme with 0.01 unit activity was used in this experiment.
[2)]IC_{50} values were determined by an inhibition assay at substrate concentration of 50 mM; [a,b]means in the same column with different superscripts are significantly different ($p<0.05$).

Inhibitory activity of bromophenols against β-glucuronidase IC_{50} values of bromophenol I and II against bacterial β-glucuronidase were 5.4 and 8.5 mg/mL, respectively, in which bromophnel I had higher inhibitory activity than bromophenol II ($p<0.05$) (Table 2). This was similar to the result of Kurihara *et al.* (25), in which α-glucosidase inhibitory potencies of the bromophenol increased with the increasing degree of bromo-substitution per benzene ring and decreasing degree of methyl-substitution. Polyphenolic compounds such as tannins from terrestrial plants and phlorotannins from marine algae are known to be associated with a variety of proteins to form complex (35), thus might resulted in exerting inhibitory activity. For example, *O*-quinones derived from catechols are covalently bound to protein amino acid and thiol groups (36). Algal β-glucuronidase inhibitor, bromophenol, belongs to phenol compounds. Therefore, bromophenols should bind to both active and non-active site of the enzymes (36). Based on the statement mentioned above, bromophenol of red algae, *G. elliptica*, is an effective inhibitor against β-glucuronidase. Bromophenol will decrease blood glucose level as well as adverse gastrointestinal effects and abdominal discomfort (37). Therefore, bromophenol can be developed as a novel natural pharmaceutical agent because of its high inhibitory activity against β-glucuronidase. Because above results were obtained *in vitro*, further study should be carried out *in vivo* for commercial application. If the structure of bromophenol is deformed in the human body by stomach acid or digestive enzymes, it is possible to be changed in its inhibitory activity.

Endo-β-D-glucuronidase (commonly referred to as heparanase) is an extracelluar matrix enzyme that degrades heparin sulfate and heparin sulfate proteoglycans, the major components of extracelluar matrix and basement membrane (38,39). Therefore, this enzyme is thought to accelerate cancer invasion and metastasis, and is expressed

to a higher extent during inflammation, angiogenesis, or malignant transformation (40). Oral administration of *L. edodes* (19), *Z. fructus* (21), and root vegetables (18) significantly inhibited fecal β-glucuronidase and tryptophanase. This was similar to the results of Gudiel-Urbano and Goni (41), in which intestinal β-glucuronidase activity decreased when seaweeds (*Undaria pinnatifida and Porphyra ternera*) were fed. Crude drug containing β-glucuronidase inhibitory activity decreased toxic side effects of the metabolite SN-38 of the anticancer agent CPT-11 (22). Direct administration of β-glucuronidase inhibitor also decreases the formation of colon cancer in animals (42). Hence, bromophenols purified from *G. elliptica*, a potent β-glucuronidase inhibitor, can be used as a novel pharmaceutical agent for the prevention and treatment of colon cancer.

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References

1. Moore WEC, Holdeman LV. Discussion of current bacteriologic investigations of the relationships of the between intestinal flora, diet, and colon cancer. Cancer Res. 35: 3418-3420 (1975)
2. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int. J. Cancer 15: 617-631 (1975)
3. Prizont R, Konigsberg N. Identification of bacterial glycosidases in rat cecal contents. Digest. Dis. Sci. 26: 773-777 (1981)
4. Simon GL, Gorbach SL. Intestinal flora in health and disease. Gastroenterology 86: 174-193 (1984)
5. Levvy GA, Conchie J. β-Glucuronidase and hydrolysis of glucuronides. pp. 301-364. In: Glucurinic Acid. Dutton GJ (ed). Academic Press, New York, NY, USA (1966)
6. SaitoY, Takano T, Rowland I. Effects of soybean oligosaccharides on the human gut microflora *in vitro* culture. Microb. Ecol. Health D. 5: 105-110 (1992)
7. Fischer LJ, Millburn P, Smith RL, Williams RT. The fate of (^{14}C) stilboestriol in the rat. Biochem. J. 100: 698-705 (1966)
8. Weisburger JH, Grantham PH, Horton RE, Weisburger EK. Metabolism of the carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide in germ free rats. Biochem. Pharmacol. 19: 151-162 (1970)
9. Sekikawa C, Kurihara H, Goto K. Inhibition of β-glucuronidase by extracts of *Chondria crassicaulis*. Bull. Fish. Sci. Hokkaido Univ. Japan 53: 27-30 (2002)
10. Hashimoto K, Saito H, Ohsawa R. Glycopolymeric inhibitors of β-glucuronidase. III. Configurational effects of hydroxyl groups in pendant glyco-units in polymers upon inhibition of β-glucuronidase. J. Polym. Sci. 44: 4895-4903 (2006)
11. Kawasaki M, Hayashi T, Arisawa M, Morita N, Berganza LH. 8-Hydroxytricetin 7-glucuronide, a β-glucuronidase inhibitor from *Scoparia dulcis*. Phytochemistry 27: 3709-3711 (1988)
12. Hayashi T, Kawasaki M, Okamura K, Tamada Y, Morita N. Scoparic acid A, a β-glucuronidase inhibitors from *Scorparia dulcis*. J. Nat. Prod. 55: 1748-1755
13. Kim DH, Shim SB, Kim NJ, Jang IS. β-Glucuronidase inhibitory activity and hepatoprotective effect of *Ganoderma lucidum*. Biol. Pharm. Bull. 22: 162-164 (1999)
14. Nawaz HR, Malik A, Khan PM, Shujaat S, Rahman AU. A novel β-glucuronidase inhibiting triterpenoid from *Paeonia emodi*. Chem. Pharm. Bull. 48: 1771-1773 (2000)
15. Di Bello IC, Dorling P, Fellows L, Winchester B. Specific inhibition of hyman β-D-glucuronidase and α-L-iduronidase by a trihydroxy pipecolic acid of plant origin. FEBS Lett. 176: 61-64 (1984)
16. Shim SB, Kim NJ, Kim DH. β-Glucuronidase inhibitory activity and hepatoprotective effect of 18-glycyrrhetinic acid from the Rhizomes of *Glycyrrhiza uralensis*. Planta Med. 66: 40-43 (2000)
17. Shim SB, Park JS, Kim NJ, Kim DH. β-Glucuronidase-inhibitory activity and hepatoprotective effect of herbal medicines. Korean J. Pharmacogn. 30: 111-114 (1999)
18. Han MJ, Kim NY. The preference and inhibitory effect of root vegetables on β-glucuronidase and tryptophanase of human intestinal bacteria. Korean J. Soc. Food Sci. 15: 555-564 (1999)
19. Han MJ, Bae EA, Kim DH. Effect of *Lentinus edodes* water extract on some enzymes of mouse intestinal bacteria. Korean J. Food Sci. Technol. 33: 142-145 (2001)
20. Zhang C, Zhang Y, Chen J, Liang X. Purification and characterization of baicalcin-β-glucuronidase hydrolyzing baicalin to baicalein from freah roots of *Scutellaria viscidula* Bge. Process Biochem. 40: 1911-1915 (2005)
21. Rhee YK, Kim DH, Han MJ. Inhibitory effect of *Zizyphi fructus* on β-glucuronidase and tryptophanase of human intestinal bacteria. Korean J. Food Sci. Technol. 30: 199-205 (1998)
22. Narita M, Nagai E, Hagiwara H, Aburada M, Yokoi T, Kamataki T. Inhibition of β-glucuronidase by natural glucuronides of *kampo* medicines using glucuronide of SN-38 (7-ethyl-10-hydroxycamptothecin) as a substrate. Xenobiotica 23: 5-10 (1993)
23. Tohda H, Kurihara H, Hosokawa M, Takahashi K. Inhibition of β-glucuronidase by purine nucleotides. Fish. Sci. 65: 667-668 (1999)
24. Kurihara H, Ando J, Hatano M. Sulfoquinovosyldacylglycerol as an alpha-glucosidase inhibitor. Bioorg. Med. Chem. Lett. 5: 1241-1244 (1995)
25. Kurihara H, Mitani T, Kawabata J, Takahashi K. Inhibitory potencies of bromophenols from Rhodomelaceae algae against α-glucosidase activity. Fish. Sci. 65: 300-303 (1999)
26. Kurihara H, Mitani T, Kawacata J, Takahashi K. Two new bromophenols from red alga *Odonthalia cormbifera*. J. Nat. Rod. 62: 882-884 (1999)
27. Yermakova SP, Sova VV, Zvyagintseva TN. Brown seaweed protein as an inhibitor of marine mollusk endo-(1→3)-β-D-glucanases. Carbohyd. Res. 227: 229-237 (2002)
28. Sugano M, Sato A, Nagak H, Yoshiok S, Shiraki T, Horikoshi H. Aldose reductase inhibitors from the red alga, asparagopsis taxiformis. Tetrahedron Lett. 31: 7015-7016 (1990)
29. Kim YC, Kim MY, Takaya Y, Niwa M, Chung SK. Phenolic antioxidants isolated from mulberry leaves. Food Sci. Biotechnol. 16: 854-857 (2007)
30. Jun M, Lee SH, Choi SH, Bae KH, Seong YH, Lee KB, Song KS. Plant phenolics as β-secretase (BACE1) inhibitors. Food Sci. Biotechnol. 15: 617-624 (2006)
31. Reese ET, Parrish FW. Nojirimycin and D-glucose-1, 5-lactone as inhibitors of carbohydrases. Carbohyd. Res. 18: 381-388 (1968)
32. Bohm BA. Introduction of flavonoids. p. 503. In: Chemistry and Biochemistry of Organic Natural Products. Ravin-dranath B (ed). Hardwood Academic Publishers, Calcutta, India (1998)
33. Suffness M, Newman DJ, Snader K. Discovery and development of antineoplastic agents from natural sources. Bioorg. Marine Chem. 3: 131-168 (1989)
34. Colgate SM, Molyneux RJ. Detection, isolation, and structural determination. p. 35. In: Bioactive Natural Products. CRC Press, Boca Raton, FL, USA (1993)
35. Stern JL, Hagerman AE, Steinberg PD, Mason PK. Phlorotannins-protein interactions. J. Chem. Ecol. 22: 1877-1899 (1996)
36. Pierpoint WS. *O*-Quinones formed in plant extracts. Their reactions with amino acids and peptides. J. Biochem. 112: 609-616 (1969)
37. Tewari N, Tiwari VK, Mishra RC, Tripathi RP, Srivastava AK, Ahmad R, Srivastava R, Srivastava BS. Synthesis and bioevaluation glycosyl ureas as alpha-glucosidase inhibitors and their effect on mycobacterium. Bioorgan. Med. Chem. 11: 2911-2922 (2003)
38. Nakajima M, Irimura T, Nicolson GL. Haparanases and tumor metastasis. J. Cell Biochem. 36: 157-167 (1988)

39. Toyoshima M, Nakajima M. Human heparanase: Purification, characterization, cloning, and expression. J. Biol. Chem. 274: 24153-24160 (1999)
40. Friedmann Y, Vlodavsky I, Aingorn H, Aviv A, Peretz T, Pecker I, Pappo O. Expression of heparanase in normal, dysplastic, and neoplastic human colonic mucosa and stroma. Evidence for its role in colonic tumorigenesis. Am. J. Pathol. 157: 1167-1175 (2000)
41. Gudiel-Urbano M, Goni I. Effect of edible seaweeds (*Undaria pinnatifida* and *Porphyra ternera*) on the metabolic activities of intestinal microflora in rats. Nutr. Res. 22: 323-331 (2002)
42. Takada H, Hirook T, Hiramatsu Y, Yamamoto M. Effect of β-glucuronidase inhibitor on azoxymethane-induced colonic carcinogenesis in rats. Cancer Res. 42: 331-334 (1982)