Supplementary Materials

Anti-inflammatory activities of black raspberry seed ellagitannins and their structural effects on the stimulation of glucagon-like peptide-1 secretion and intestinal bitter taste receptors

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Table S1. Primer sequences used in real-time quantitative polymerase chain reaction analysis

Gene	Forward (5'→3')	Reverse (5'→3')
Actb	ACAGCTTCTTTGCAGCTCCTTCG	ATCGTCATCCATGGCGAACTGGTG
Ocln	TCACTTTTCCTGCGGTGACT	GGGAACGTGGCCGATATAATG
Cldn1	GGCTTCTCTGGGATGGATCG	CCCCAGCAGGATGCCAATTA
Tjp1	TCTTCCATCATTTCGCTGTGT	TCTGAAACCATCAAGTCCACA
Gcg	TGTCTACACCTGTTCGCAGC	TCCTCATGCGCTTCTGTCTG
Glp1r	GTGGCTATCCTGTACTGCTTTGTC	CTTCATGCTGCAGTCTCTCTGG
mTas2r108	ACAGTCGCAGAATTGCCTCTCC	AGGAATCTAGTGATGGCCAAGCTG
mTas2r113	TCCGCACTGCTCTGGCAATTAG	TGAACAGACACCCACCAATCTAGG
mTas2r118	AAGTTGCACAACGGTTGCAGTG	TCTCCACCGGTGACAGTCTTTG
mTas2r119	CTACTGTGCCAAGATTGCTACC	GGTCTGACCCGAGTTGTATTTTT
mTas2r125	ATCTTCTCCCTGTGGAGACACCTG	TGGTGTCTTCGGAGCCTTTAGC
mTas2r126	GCAGTGTGTGGGATTGGTCAAC	TCCCGGAGTACTCAACCAGATG
mTas2r131	ATCAACATGGCTTGCCACCTG	AGCACACCTCTCAATCTCCACTTC
mTas2r135	CAGCCTCTCGATTCTGTCTCC	AGGCAACCTGTACTTTAGCCA
mTas2r136	TCTGGAGGAACCAATCCACCTG	TGCTCTCACCTGAACCATTGCC
mTas2r137	AGCATACATTTGTGGCCATGCTC	AAGCAGAGGGTCCCTTAGATCCAG
mTas2r138	TCATTTCTGTTCCTTTCAGCCAT	CAGCAGTGCGATGTCACAGT
mTas2r140	CATGCAACACAATGCCAAAGACTC	AGGGCCTTAATATGGGCTGTGG
mTas2r143	TTCCCAGGCTGCTGGTTGTATC	AGTTCCCGGTGGCTGAAATGAC

Actb, *β*-actin gene; *Gcg*, preproglucagon gene; *Glp1r*, glucagon-like peptide-1 receptor gene; *Ocln*, occludin gene; *Cldn1*, claudin-1 gene; *Tjp1*, zonula occludens-1 gene; *mTas2r*, mouse bitter taste receptor gene.

Table S2. Predicted binding energies (ΔG, kcal/mol) of ligands as positive controls against the predicted structure of mouse bitter taste receptor subtype 108 (mTAS2R108)

Compound	ΔG against the mTAS2R108 model
Amarogentin	-9.371
Artemorin	-7.873
Chlorpheniramine	-7.232
Diphenidol	-8.003
Emetine	-8.879
6-Propyl-2-thiouracil	-4.927
Yohimbine	-8.04

All structural files of the compounds were downloaded from BitterDB (https://bitterdb.agri.huji.ac.il/dbbitter.php).

Table S3. Predicted binding energies (ΔG, kcal/mol) of sweet-tasting compounds against the predicted structure of mouse bitter taste receptor subtype 108 (mTAS2R108)

Compound	ΔG against the mTAS2R108 model
Acesulfame-K	-4.891
Aspartame	-6.509
Fructose	-4.163
Glucose	-4.092
Saccharin	-5.973
Sucralose	-5.927
Sucrose	-5.487

Table S4. Ellagitannins in ellagitannin fraction from black raspberry seeds, identified by UHPLC-Q-TOF-MS/MS

Peak	MS data (*m/z*)	MS/MS data (*m/z*)	Proposed compound	Reference number
1	**933.0681**, 466.0285	933.0724, 631.0670, 569.0583, 301.0042	Vescalagin**	1
2	1568.1533, **783.0706**	783.0693, 481.0632, 300.9986	Pedunculagin isomer	2 and 3
3	**933.0663**, 466.0280	933.0728, 631.0568, 569.0520, 300.9948	Castalagin**	2 and 4
4	1568.1470, **783.0707**	783.0718, 481.0647, 300.9996	Pedunculagin*	2 and 3
5	1205.1318, **602.5634**	915.0657, 602.0667,457.0237, 289.0717	Acutissimin A*	5
6	1568.1486, **783.0688**	1236.0748, 935.0851, 783.5720, 633.0776, 469.0094, 300.9991	Sanguiin H-10 isomer	3, 6, 7, and 8
7	1717.1426, **858.0655**, 577.1318	1235.0795, 1113.1243, 933.0635, 783.0630, 633.0742, 481.0637, 300.9987	Sanguiin H-6 isomer without galloyl moiety	6 and 8
8	**1207.1564**, 603.0723	1207.1626, 917.0737, 573.0535,	Stenophyllanin A	9
9	**935.0798**, 467.0363	935.0860, 783.0750, 633.0779, 300.9966	Casuarictin isomer	3 and 10
10	1103.0898, **551.0402**	1103.0919, 935.0858, 633.0811, 469.0060, 300.9984	Sanguiin H-2 isomer	3 and 8
11	1568.1445, **783.0695**	1235.0931, 935.0810, 783.0785, 633.0848, 300.9991	Sanguiin H-10 isomer	3, 6, 7, and 8
12	1870.1530, **934.5761**	1567.1794, 1235.0912, 934.5823, 633.0816, 300.9960	Sanguiin H-6 isomer	3, 6, 7, and 8
13	**935.5840**, 467.0361	935.0840, 783.0773, 633.0732, 300.9986	Casuarictin**	3 and 10
14	1870.1644, **934.5779**	1567.1692, 1235.0779, 934.5799, 633.0771, 301.0010	Sanguiin H-6*	3, 6, 7, and 8
15	**935.5818**, 467.0354	935.0842, 783.0933, 633.0805, 301.0012	Casuarictin isomer	3 and 10

Bold letters of MS data are precursor ions of MS/MS data. The peak numbers are the same as in Fig. S1. *Compounds were identified by nuclear magnetic resonance spectroscopy. **Compounds were identified by comparison with standards. All the numbered references are listed at the end of the Supplementary materials.

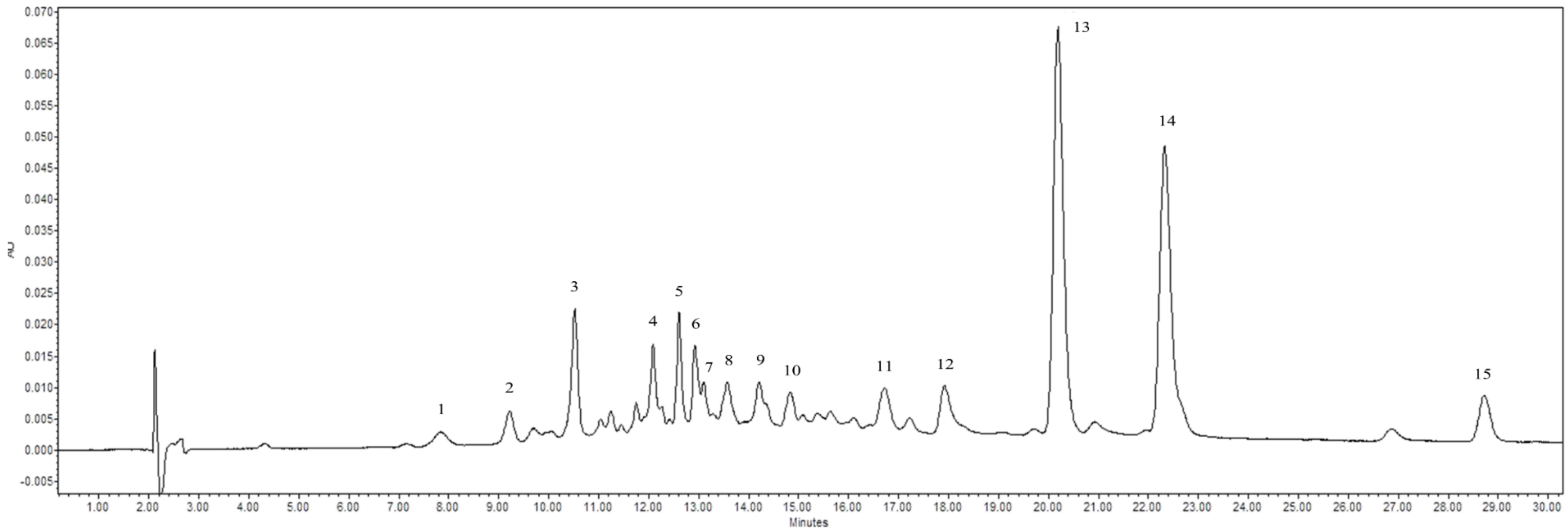

Fig. S1. Chromatogram of ellagitannins in ellagitannin fraction from black raspberry seeds. The peaks represent 1, vescalagin; 2, pedunculagin isomer; 3, castalagin; 4, pedunculagin; 5, acutissimin A; 6, sanguiin H-10 isomer; 7, sanguiin H-6 isomer without galloyl moiety; 8, stenophyllanin A; 9, casuarictin isomer; 10, sanguiin H-2 isomer; 11, sanguiin H-10 isomer; 12, sanguiin H-6 isomer; 13, casuarictin; 14, sanguiin H-6; and 15, casuarictin isomer.

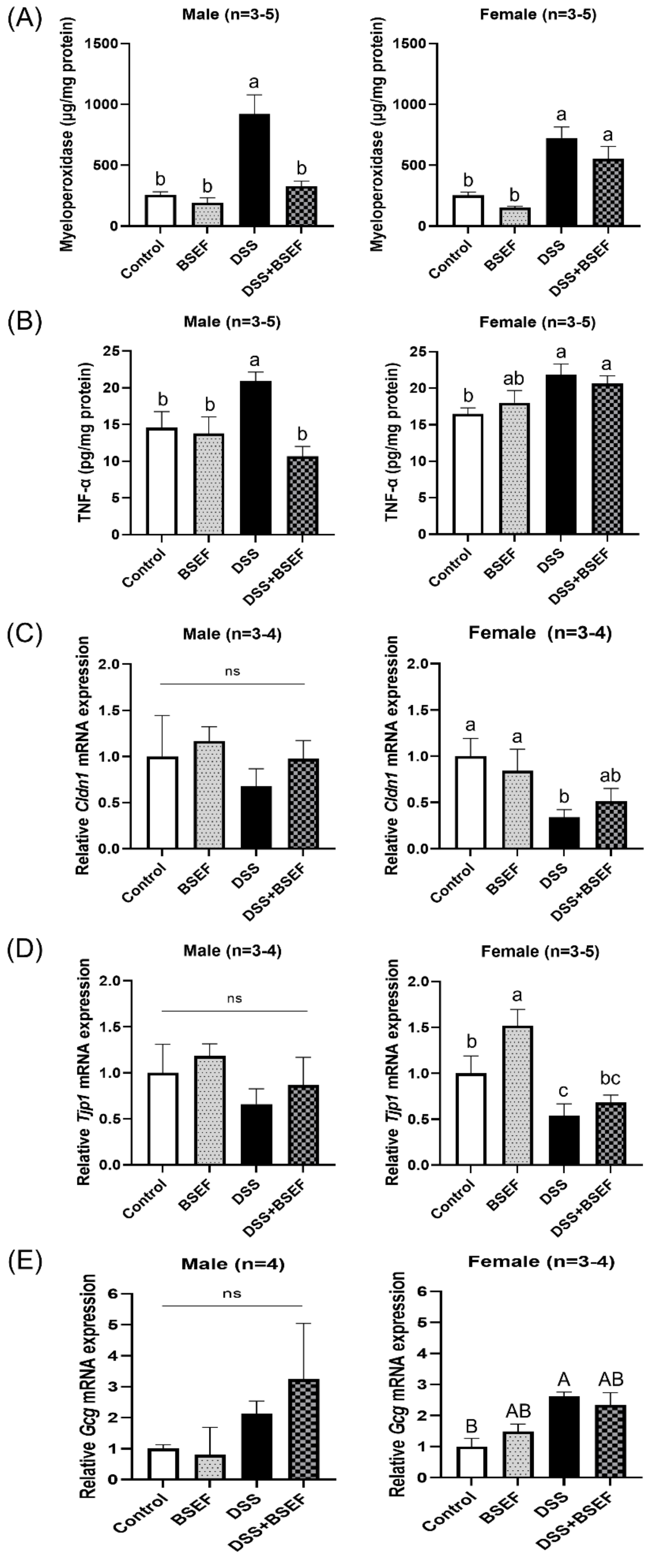
(A)
Male (n=3-5)
Myeloperoxidase (µg/mg protein)
1500
1000
500
0
a
b
b
b
Control
BSEF
DSS
DSS+BSEF
Female (n=3-5)
Myeloperoxidase (µg/mg protein)
a
a
b
b
(B)
Male (n=3-5)
TNF-α (pg/mg protein)
25
20
15
10
5
0
a
b
b
b
Female (n=3-5)
TNF-α (pg/mg protein)
b
ab
a
a
(C)
Male (n=3-4)
Relative Cldn1 mRNA expression
2.0
1.5
1.0
0.5
0.0
ns
Female (n=3-4)
Relative Cldn1 mRNA expression
a
a
b
ab
(D)
Male (n=3-4)
Relative Tjp1 mRNA expression
ns
Female (n=3-5)
Relative Tjp1 mRNA expression
b
a
c
bc
(E)
Male (n=4)
Relative Gcg mRNA expression
6
5
4
3
2
1
0
ns
Female (n=3-4)
Relative Gcg mRNA expression
B
AB
A
AB

Fig. S2. Sex differences in the colonic level of myeloperoxidase (A), tumor necrosis factor-α (TNF- α) (B), *Cldn1* (claudin-1 gene) (C), *Tjp1* (zonula occludens-1 gene) (D), and *Gcg* (preproglucagon gene) (E) in C57BL/6J mice with dextran sulfate sodium (DSS)-induced colitis. All data represent the means and standard errors of means. Relative gene expressions were normalized to *Actb* expression. BSEF, supplemented with ellagitannin fraction from black raspberry seeds by oral gavage (333 mg/kg body weight/day). DSS, administered with 1.5% (w/v) DSS-containing water *ad libitum*. Control and DSS groups were provided with normal saline by oral gavage. (A-D) Different lower cases indicate significant differences among the groups ($p<0.05$; one-way ANOVA and Duncan's multiple range test). (E) Different upper cases indicate significant differences among the groups ($p<0.05$; Kruskal-Wallis test with Dunn's test). ns, not significant.

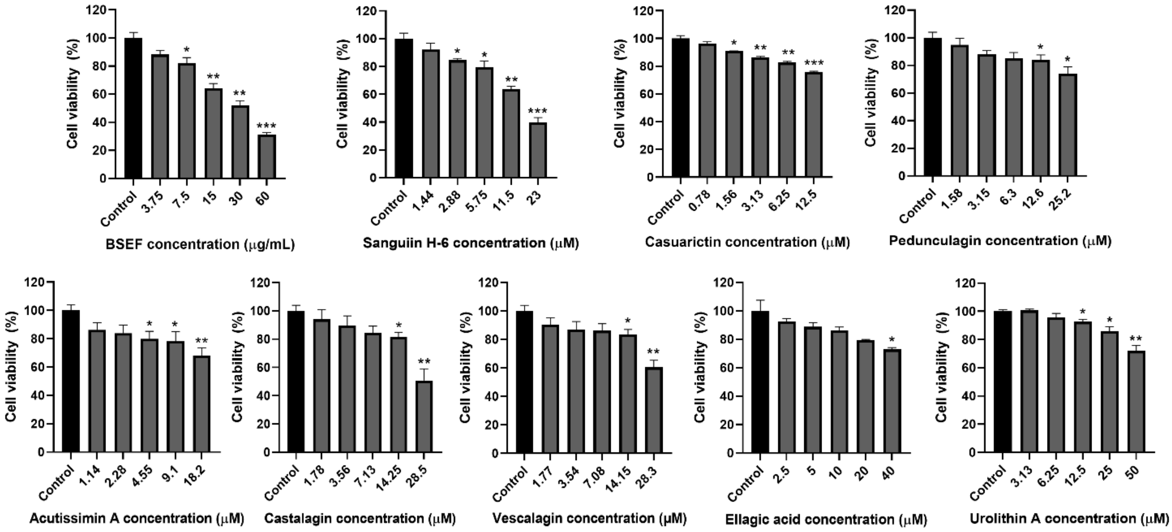

Fig. S3. Viability of STC-1 cells treated with different concentrations of ellagitannin fraction from black raspberry seeds (BSEF), six ellagitannins in BS, and their metabolites (ellagic acid and urolithin A) (n=3-4). All data represent the means and standard errors of means. Control group was treated with serum-free Dulbecco's modified Eagle medium containing 0.5% dimethyl sulfoxide. *p<0.05, **p<0.01, and ***p<0.001 by independent t-test compared with the control.

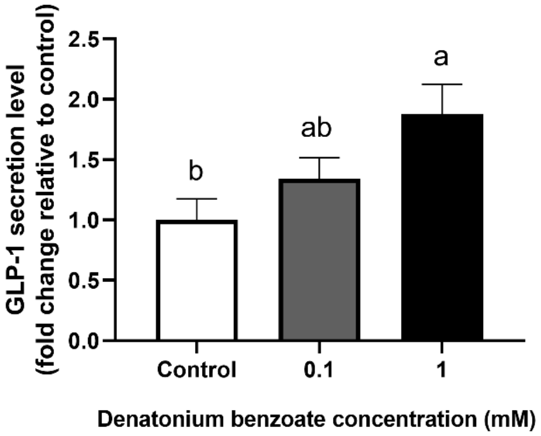

Fig. S4. Glucagon-like peptide-1 (GLP-1) secretion induced by treatment of denatonium benzoate, used as a positive control, in STC-1 cells (n=3-4). All samples were treated for 2 h. All data represent the means and standard errors of means. Control group was treated with Hanks' balanced salt solution containing 0.1% (w/v) bovine serum albumin. Values with different letters are significantly different (p<0.05; one-way ANOVA and Duncan's multiple range test).

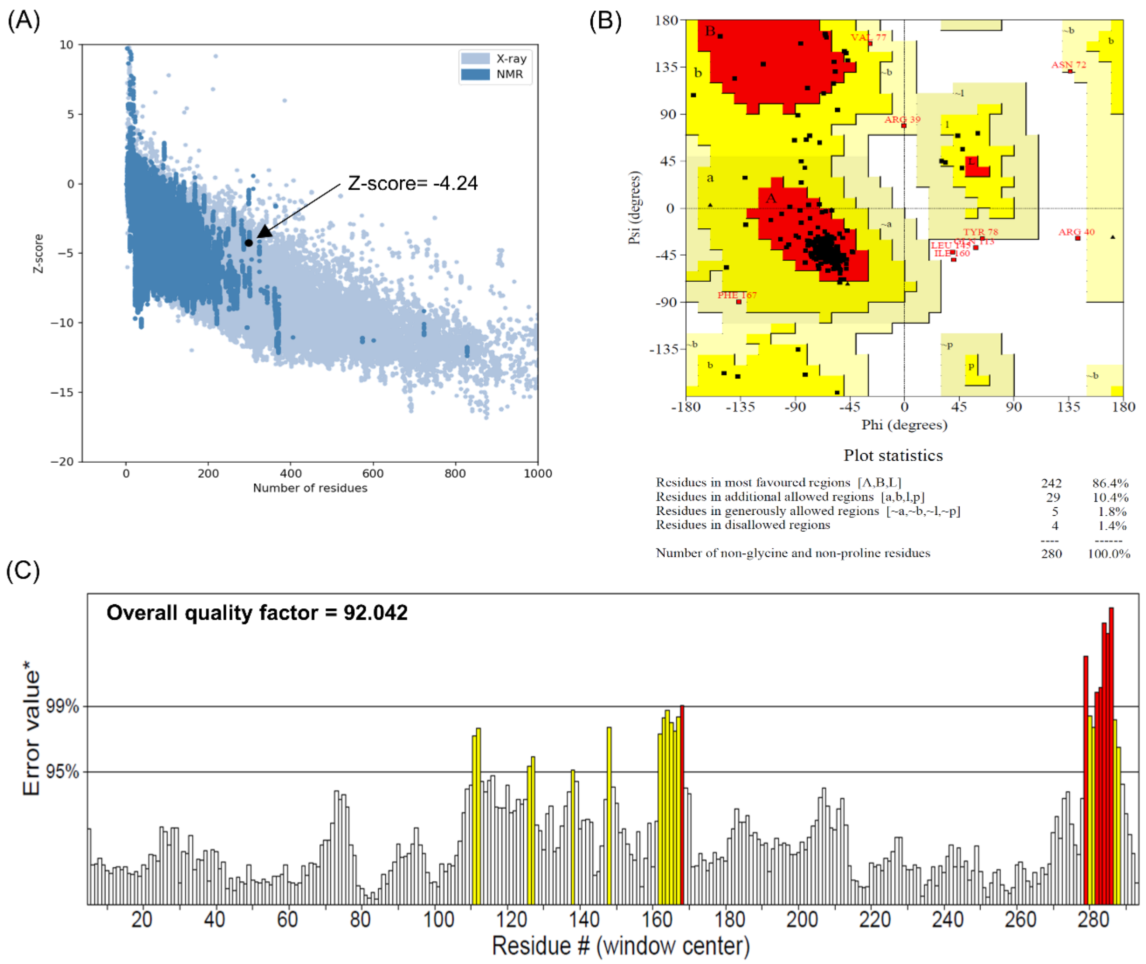

Fig. S5. Structural validation result of the predicted three-dimensional structure of mouse bitter taste receptor subtype 108 (mTAS2R108). (A) The z-score of the mTAS2R108 model, which was calculated by ProSA, was within the z-score range of native proteins having similar size with mTAS2R108 in Protein Data Bank library. (B) Ramachandran plot provided by PROCHECK was used to evaluate whether backbone dihedral angles (phi and psi angles) in a polypeptide chain of the mTAS2R108 model were stereochemically possible. (C) ERRAT was used to examine non-covalently bonded atomic interaction between amino acids in the mTAS2R108. Overall quality factor was expressed as the percentage of amino acids whose error values were below 95% confidence limit out of total amino acids in the mTAS2R108 model.

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