Amyloid Polypeptide Disaggregation Activity of Passion Fruit Seed-Derived Polyphenol Compounds

Tatsuya Sampei¹, Yingxue Wu¹ and Hideyuki Shigemori²,3

Abstract
In an aging society, the prevalence of Alzheimer disease (AD) and type 2 diabetes (T2D) has increased. It is currently hypothesized that these diseases are caused by the aggregation of amyloid β (Aβ) in the brain and human islet amyloid polypeptide (hIAPP) in the islets of Langerhans, respectively. Therefore, the disaggregation of these existing amyloid aggregates is a promising approach to the prevention and treatment of both diseases. In our previous studies, we found a remarkable Aβ and hIAPP aggregation inhibitory activity of polyphenolic compounds containing catechol moieties. Compared to previous reports on their aggregation inhibitory activity, there are few on the disaggregation activity of polyphenolic compounds. Additionally, there are few findings on the disaggregation activity of polyphenolic compounds on hIAPP. In this study, we investigated the Aβ and hIAPP disaggregation activity of scirpusin B, a polyphenolic compound found in passion fruit seeds, and related compounds. Thioflavin T (Th-T) assays and transmission electron microscopy (TEM) were performed on these compounds to evaluate their Aβ42 and hIAPP disaggregation activities. The results showed that scirpusin B and its related compounds showed remarkable disaggregation activity. The structure–activity relationship of these compounds revealed that the presence of catechol moieties is important for this activity. This study also showed that polyphenols from passion fruit seeds have significant disaggregation activity against amyloid polypeptide aggregation.

Keywords
alzheimer disease, type 2 diabetes, amyloid β, human islet amyloid polypeptide, scirpusin B, structure–activity relationship, catechol, disaggregation

Received: December 27th, 2021; Accepted: March 17th, 2022.

In an aging society, the increasing number of patients suffering from dementia has become a serious concern. Alzheimer disease (AD), an intractable neurodegenerative complaint, accounts for more than half of all dementia cases. Neurodegenerative diseases, such as AD, interfere with the daily activities of patients, who are in need of long-term care and become an increasing burden on their families. Although research is underway to develop a cure, no effective treatment nor drug has been found to fundamentally treat AD itself. Similar to AD, type 2 diabetes mellitus (T2D), which is on a rise across the globe, is also a major problem. T2D is characterized by decreased insulin function, which leads to high blood glucose levels and various complications. Previous studies have shown a relationship between AD and T2D.¹ AD and T2D share many common pathophysiological characteristics, such as increased oxidative stress and the aggregation of amyloid proteins with intermolecular β-sheet structures.²–⁴ Amyloid proteins include amyloid β (Aβ) and human islet amyloid polypeptide (hIAPP).⁵–⁷ Aβ, which consists of 36 to 43 amino acids, is produced from amyloid precursor protein in the brain, and hIAPP, which consists of 37 amino acids, is secreted from pancreatic β-cells.⁸ These aggregates attack cells in various ways.⁹ For example, they stimulate the production of cytotoxic molecules, such as nitric oxide, reactive oxygen species (ROS), and pro-inflammatory cytokines in glial cells, contributing significantly to neuronal damage and death.¹⁰,¹¹ It has also been proposed that these aggregates load unfolded-protein response pathways,¹² which leads to cerebral and hippocampal atrophy in the brain and insulin deficiency in the pancreas. Furthermore, recent studies have shown that hIAPP is mixed with senile plaques, which are

¹Graduate School of Science and Technology, University of Tsukuba, Tsukuba, Ibaraki, Japan
²Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan
³Microbiology Research Center for Sustainability (MiCS), University of Tsukuba, Tsukuba, Ibaraki, Japan

Corresponding Author:
Hideyuki Shigemori, Microbiology Research Center for Sustainability (MiCS), University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.
Email: shigemori.hideyuki.fn@u.tsukuba.ac.jp
Aβ aggregates found specifically in the brains of patients with AD. Additionally, Aβ has been found to aggregate in the pancreas of transgenic mice expressing both Aβ and hIAPP. Therefore, the disaggregation of these toxic oligomeric and fibrillar species may prove important for the treatment of AD and T2D. However, there is no effective therapy that can reverse the formation of these aggregates. Finding a compound that can disaggregate both the amyloid proteins would therefore be an effective agent for the prevention and treatment of both diseases.

Recently, plant extracts, and especially flavonoids, have been reported to exhibit disaggregating activity. In addition, resveratrol and related compounds have been reported to inhibit the aggregation of Aβ protein and hIAPP, which has been

Figure 1. Molecular structures of compounds 1–7.
attracting significant attention.\textsuperscript{17,18} However, there are no reports on resveratrol analogs. Nonetheless, it is important to investigate the disaggregation activities of both hIAPP and \( \text{A}\beta \) amyloid polypeptides. In our previous study, we found that polyphenols derived from various natural products inhibit amyloid polypeptide aggregation.\textsuperscript{19–27} In this study, we evaluated the disaggregation activities of scirpusin B and its related compounds from passion fruit seeds.

Figure 2. Effect of compounds 1–7 on \( \text{A}\beta 42 \) disaggregation. \( \text{A}\beta 42 \) (25 \( \mu \)M) fibril formation was monitored by Th-T fluorescence after treatment with 1, 5, and 10 \( \mu \)M of each compound. Fluorescence intensity was measured at excitation and emission wavelengths of 420 nm and 485 nm, respectively. Values represent the mean ± SD (\( n = 6 \)).
against amyloid polypeptide aggregation and expounded a structure–activity relationship for these compounds.

**Materials and Methods**

**Tested Compounds 1–7**

*trans*-scirpusin B (1), *cis*-scirpusin B (2), *trans*-scirpusin A (3), *trans*-piceatannol (4), *cis*-piceatannol (5), and resveratrol (6) were purchased from Nagara Science Co. Ltd, Japan, and *trans*-tetramethylpiceatannol (7) from FUJIFILM Wako Pure Chemical Corporation, Japan.

**Thioflavin T (Th-T) Assay**

The disaggregation abilities of Aβ42 and hIAPP were evaluated using the Th-T method developed by Naiki et al.28 Herein, Aβ42 was dissolved in 0.1% NH4OH or hIAPP (KareBay Biochem Inc) dissolved in a 250 mM solution of 1,1,1,3,3,3-hexafluoro-2-propanol (0.5% acetic acid aqueous solution). The amyloid polypeptide solution was then diluted 10-fold with 50 mM PBS (pH = 7.4) and incubated with or without compounds 1–7 (Figure 1). The amyloid polypeptide solution (2.5 µL) was then added to 250 µL of 1 mM Th-T in 50 mM Gly-NaOH solution (pH = 8.5). The amyloid polypeptides were pre-incubated for 24 h to form aggregates beforehand, and then compounds 1–7 were added. The fluorescence intensities were measured at excitation and emission wavelengths of 420 nm and 485 nm, respectively, using a Wallac 1420 ARVO MX Multidetection Microplate Reader (PerkinElmer). The IC50 value of each compound was calculated from the inhibition rate (%) of amyloid polypeptide aggregation after 24 h of incubation at 37 °C.

**Transmission Electron Microscope (TEM) Observations**

The procedure was performed as previously reported28 but with slight modifications. First, after the Th-T assay, 5 µL of the amyloid polypeptide sample was spotted onto a glow-discharge carbon-coated Formvar grid, incubated for 2 min, then washed twice with 5 µL of distilled water. The resulting grid was negatively stained twice for 1 min each with 5 µL of 0.4% silicotungstic acid. After air-drying for 10 min, the samples were analyzed by transmission electron microscopy (TEM) (JEOL, JEM-1400).

**Results**

**Aβ42 Disaggregation Activity of Compounds 1–7**

Th-T fluorescence assays were performed on compounds 1–7 to evaluate their Aβ42 disaggregation activities (Figure 2). The IC50 values of these compounds are listed in Table 1. Compounds 1 (IC50 = 1.0 µM) and 2 (IC50 = 1.3 µM) exhibited significant Aβ disaggregation activity, compounds 5 (IC50 = 2.1 µM), 4 (IC50 = 1.9 µM), and 3 (IC50 = 2.1 µM) showed moderate Aβ disaggregation activity, compound 6 (IC50 = 7.7 µM) showed low disaggregation activity, and compound 7 (IC50 > 100 µM), which contained no catechol moieties, exhibited no disaggregation inhibitory activity (Table 1).

| Compounds | aIC50 value (µM) | Aβ and hIAPP |
|-----------|-----------------|--------------|
| trans-scirpusin B (1) | 1.0 and 3.0 | |
| cis-scirpusin B (2) | 1.3 and 3.0 | |
| trans-scirpusin A (3) | 2.1 and 3.2 | |
| trans-piceatannol (4) | 1.9 and 3.8 | |
| cis-piceatannol (5) | 2.1 and 4.4 | |
| resveratrol (6) | 7.7 and 31.0 | |
| trans-tetramethylpiceatannol (7) | >100 and >100 | |

The IC50 values were calculated from the inhibitory rate (%) of each concentration of derivatives for amyloid polypeptide aggregation estimated using the Th-T assay after 24 h.
significant Aβ disaggregation activity, and compounds 3 (IC_{50} = 3.2 µM), 4 (IC_{50} = 3.8 µM), and 5 (IC_{50} = 4.4 µM), which contain only one catechol moiety each, showed a lower disaggregation activity than compounds 1 and 2. Furthermore, compounds 6 (IC_{50} = 31.0 µM) and 7 (IC_{50} >100 µM), which have no catechol moieties, were less active than compounds 1 and 2. Compound 7 did not exhibit any disaggregation activity.

To verify the effects of compounds 1–7 on hIAPP fiber formation, direct TEM observations were performed (Figure 5). Conducting the experiment with only hIAPP showed that the hIAPP fibers spread in a mesh pattern. It was confirmed that the addition of compounds 1–7 (10 µM) suppressed hIAPP fiber formation to varying degrees. As far as the structure–activity relationship is concerned, it was found that compounds 1 and 2, which contain 2 catechol moieties each, exhibited significantly disaggregation activity against the formation of hIAPP fibers. Compounds 3, 4, and 5, each having a catechol moiety, were found to have moderate disaggregation activity against hIAPP fiber formation. Compounds 6 and 7 were found to be less active and showed a greater disaggregation activity than the other compounds. These results were consistent with those of the Th-T assay.

**Discussion**

In the present study, we investigated the effects of trans-scirpusin B (1) and its related compounds 2–7 on Aβ42 and hIAPP aggregation. Th-T assays were utilized to evaluate the disaggregation activities of compounds 1–7 on Aβ42 and hIAPP. Compounds 1 and 2 showed strong disaggregation activity against Aβ42 and hIAPP aggregation. While compound 4, which contains one catechol moiety, showed disaggregation activity against Aβ and hIAPP aggregation in the Th-T assay, its activity was lower than that of compound 1, which contains 2 catechol moieties. Furthermore, compounds 6

---

**Figure 3.** Effects of compounds 1–7 on Aβ42 fibrillogenesis identified using a TEM. Fibril formation was observed after 24 h of incubation in a 50 µM PBS buffer. Scale bars: 1 µm. **A:** Aβ42 (25 µM), **B:** Aβ42 (25 µM) + 1 (10 µM), **C:** Aβ42 (25 µM) + 2 (10 µM), **D:** Aβ42 (25 µM) + 3 (10 µM), **E:** Aβ42 (25 µM) + 4 (10 µM), **F:** Aβ42 (25 µM) + 5 (10 µM), **G:** Aβ42 (25 µM) + 6 (100 µM), and **H:** Aβ42 (25 µM) + 7 (100 µM).
and 7, which had no catechol moieties, were less active than compounds 1–5. The structure–activity relationship showed that steric differences did not affect the extent of activity.

In conclusion, the compounds with catechol moieties exhibited better disaggregation activity. The degree of activity was related to the number of catechol moieties present in the compounds, which suggests that catechol moieties affect Aβ and hIAPP structure. These results (Table 1) are consistent with our previous studies on aggregation inhibition.19–27 The following mechanisms were proposed based on the experimental
results. First, the catechol moiety is autoxidized to o-benzoquinone. The autoxidation of the catechol moiety to o-benzoquinone is thought to result in a Michael addition of basic amino acid residues to Aβ, in particular to the Lys16 and Lys28, resulting in structural changes of the protein. Furthermore, the benzene ring of the compounds employed in this experiment may cause protein conformational changes by inducing π-π stacking, according to the orientation of the amino acid residues of Aβ. This π-π stacking may destabilize the fibrils by disrupting their β-sheet structure, which is thought to be necessary for aggregation. According to literature, Aβ42 and hIAPP are structurally similar and may destabilize the fibril structure through a similar mechanism.

Second, small molecules such as brazilin and polyphenols have also been reported to disaggregate amyloid fibrils into non-toxic amyloid aggregates. The exact mechanism is unclear, but it has been suggested that direct interaction of natural compounds with the fibril β-sheet is important for understanding the dissociation mechanism. Molecular dynamics simulations show that hydrogen bonds are formed with Asp23 within the fibrils and there is binding to the intermolecular Asp23-Lys28 salt bridge, which is important for stabilizing amyloid fibrils. This destabilizes the hydrogen bonds in the amino acid backbone that supports the fibrils, resulting in fibril remodeling and disaggregation. Amentoflavone-type bioflavonoids have been shown to bind preferentially to the N-terminus of fibrils via π-π interactions, resulting in the disaggregation of Aβ fibrils. The aromatic ring of the compound binds to the aromatic residues of the fibrils and fits into the N-terminus pocket, thereby stabilizing the bioflavonoid-fibril complex. Hydrogen bonds are subsequently formed between the hydroxyl groups of the compound and the peptide backbone, significantly reducing the β-sheet content of the fibrils and altering the Aβ fibril conformation, leading to the fibril disaggregation.

Since the compound used in this experiment is a small molecule with a catechol moiety, a structure similar to that of brazilin, it may disaggregate via the mechanism described above.

We also compared the disaggregation and anti-aggregation activities of Aβ42 and hIAPP and found that Aβ42 and hIAPP showed similar behavior. These results support the results obtained in our previous studies that polyphenols show comparable results in both Aβ42 and hIAPP disaggregation activity.

In the present study, Aβ42 and hIAPP disaggregation activity tests using trans-scirpusin B (1) and its related compounds 2–
The active compounds 1–5 can be used to prevent and/or treat AD and T2D, while they have different inhibitory activities.

Acknowledgments

The authors thank Professor Kazuhiro Irie, Associate Professor Kazuma Murakami, Dr. Mizuho Hanaki, and Dr. Yumi Irie, Graduate School of Agriculture, Kyoto University for preparing APβ42. The TEM observation method was used with a JEOL JEM-1400 at the Center for Medical Electron Microscopy, University of Tsukuba. The authors would like to thank Editage (www.editage.com) for English language editing.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Japan Society for the Promotion of Science (grant number JP20H05581).

ORCID iD

Hideyuki Shigemori https://orcid.org/0000-0001-9778-8057

References

1. Janson J, Laedrid T, Parisi JE, O’Brien P, Petersen RC, Butler PC. Increased risk of type 2 diabetes in Alzheimer disease. Diabetes. 2004;53(2):474–481. doi:10.2337/diabetes.53.2.474
2. Barbagallo M, Dominguez L. Type 2 diabetes mellitus and Alzheimer’s diseases. World J Diabetes. 2014;5(6):889–893. doi:10.4239/wjd.v5i5.889
3. Blázquez E, Velizquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and Alzheimer’s disease. Front Endocrinol. 2014;5(161):1–21. doi:10.3389/fendo.2014.00161
4. Takeda S, Sato N, Rakugi H, Morishita R. Molecular mechanisms linking diabetes mellitus and Alzheimer’s disease: beta amyloid peptide, insulin signaling, and neuronal function. Mol Biotechnol. 2011;7(6):1822–1827. doi:10.1039/c0mb00302f
5. Johnson KH, O’Brien TD, Hayden DW, et al. Immunolocalization of islet amyloid polypeptide (IAPP) in pancreatic beta cells by means of peroxidase-antiperoxidase (PAP) and protein A-gold techniques. Am J Pathol. 1998;130(1–2):318–326. PMID: 10921511
6. Masters CL, Simons G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and down syndrome. Proc Natl Acad Sci U S A. 1985;82(12):4245–4249. doi:10.1073/pnas.82.12.4245
7. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer’s amyloid beta peptide. Nat Rev Mol Cell Biol. 2007;8(2):101–112. doi:10.1038/nrm2101
8. Cooper GJ, Leighton B, Dimitriadis GD, et al. Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. Proc Natl Acad Sci U S A. 1998;95(20):11225–11227. doi:10.1073/pnas.95.20.11225
9. Yasayoge SA, Langen R. Membrane interaction of islet amyloid polypeptide. Biochim Biophys Acta. 2007;1768(8):2002–2009. doi:10.1016/j.bbamem.2007.01.022
10. Yang SG, Wang WY, Ling TJ, et al. Alpha-tocopherol quinone inhibits beta-amyloid aggregation and cytotoxicity, disaggregates preformed fibrils and decreases the production of reactive oxygen species, NO and inflammatory cytokines. Neurochem Int. 2010;57(8):914–922. doi:10.1016/j.neuint.2010.09.011
11. Heneka MT, O’Banion MK. Inflammatory processes in Alzheimer’s disease. J Neuroimmunol. 2007;184(1–2):69–91. doi:10.1016/j.jneuroim.2006.11.017
12. Naiki H, Geijo F. Kinetic analysis of amyloid fibril formation. Methods Enzymol. 1999;309(20):305–318. doi:10.1074/0076-6879(99)09022-9
13. Oskarsson ME, Paulsson JF, Schultz SW, Ingelsson M, Westermark P, Westermark GT. In vivo seeding and cross-seeding of localized amyloidosis a molecular link between type 2 diabetes and Alzheimer disease. Am J Pathol. 2015;185(3):834–846. doi:10.1016/j.ajpath.2014.11.016
14. Wijesekara N, Ahrens R, Sabale M, et al. Amyloid-β and islet amyloid pathologies link Alzheimer’s disease and type 2 diabetes in a transgenic model. FASEB J. 2017;31(12):5409–5418. doi:10.1096/fj.201704313R
15. Jae Eun L, Nayeon K, Ji Y, et al. Anti-amyloidogenic effects of asarone derivatives from Perilla frutescens leaves against beta-amyloid aggregation and nitric oxide production. Molecules. 2019;24(23):4297. doi:10.3390/molecules24234297
16. Windsor PK, Plassmeyer SP, Mattock DS, et al. Bilavonoid-induced disruption of hydrogen bonds leads to amyloid disaggregation. Int J Mol Sci. 2021;22(6):2888. doi.org/10.3390/ijms22062888
17. Rivière C, Papastamoulis Y, Fortin P-Y, et al. New stilbene dimers against amyloid fibril formation. Bioorg Med Chem. 2010;20(11):3441–3443. doi:10.1016/j.bmc.2009.09.074
18. Mishra R, Sellin D, Radovan D, Gohlike A, Winter R. Inhibiting islet amyloid polypeptide fibril formation by the red wine compound resveratrol. ChemBioChem. 2009;10(3):445–449. doi:10.1002/cbic.200800762
19. Miyamae Y, Kurisu M, Murakami K, et al. Protective effects of caffeoylquinic acids on the aggregation and neurotoxicity of the 42-residue amyloid β-protein. Bioorg Med Chem. 2012;20(19):5844–5849. doi:10.1016/j.bmc.2012.08.001
20. Kurisu M, Miyamae Y, Murakami K, et al. Inhibition of amyloid β aggregation by acetoside, a phenylethanoid glycoside. Biosci Biotechnol Biochem. 2013;77(6):1329–1332. doi:10.1271/bbb.130101
21. Hmielene AB, Hanaki M, Murakami K, Irie K, Isoda H, Shigemori H. Inhibitory activities of antioxidant flavonoids from Tamarix
gallica on amyloid aggregation related to Alzheimer’s and type 2 diabetes diseases. *Biol Pharm Bull*. 2017;40(2):238-241. doi:10.1248/bph.16-00801

22. Jiang G, Takase M, Aihara Y, Shigemori H. Inhibitory activities of kukoamines A and B from Lycii Cortex on amyloid aggregation related to Alzheimer’s disease and type 2 diabetes. *J Nat Med*. 2020;74(1):247-251. doi:10.1007/s11418-019-01337-0

23. Sun J, Jiang G, Shigemori H. Inhibitory activity on amyloid aggregation of rosmarinic acid and its substructures from Isodon japonicas. *Nat Prod Commun*. 2019;14(5):1-5. doi:10.1177/1934578X19843039

24. Sun J, Murata T, Shigemori H. Inhibitory activities of phenylpropanoids from Lycopus lucidus on amyloid aggregation related to Alzheimer’s disease and type 2 diabetes. *J Nat Med*. 2020;74(3):579-583. doi:10.1007/s11418-020-01398-6

25. Tsunoda T, Takase M, Shigemori H. Structure-activity relationship of clovamide and its related compounds for the inhibition of amyloid β aggregation. *Bioorg Med Chem*. 2018;26(12):3202-3209. doi:10.1016/j.bmc.2018.04.044

26. Nomoto D, Tsunoda T, Shigemori H. Effects of clovamide and its related compounds on the aggregations of amyloid polypeptides. *J Nat Med*. 2021;75(2):299-307. doi:10.1007/s11418-020-01467-w

27. Tanaka T, Betkekar VV, Ohmori K, Suzuki K, Shigemori H. Evaluation of amyloid polypeptide aggregation inhibition and disaggregation activity of A-type procyanidins. *Pharmaceuticals*. 2021;14(11):1118. doi.org/10.3390/phi14111118

28. Murakami K, Irie K, Morimoto A, et al. Neurotoxicity and physicochemical properties of Aβ mutant peptides from cerebral amyloid angiopathy. *J Biol Chem*. 2013;288(46):46179-46187. doi:10.1074/jbc.M301874200

29. Bittner S. When quinones meet amino acids: chemical, physical and biological consequences. *Amino Acids*. 2006;30(3):205-224. doi:10.1007/s00726-005-0298-2

30. Sato M, Murakami K, Uno M, et al. Site-specific inhibitory mechanism for amyloid B42 aggregation by catechol-type flavonoids targeting the Lys residues. *J Biol Chem*. 2013;288(32):23212-23224. doi:10.1074/jbc.M113.464222

31. Ishii T, Mori T, Tanaka T, et al. Covalent modification of proteins by green tea polyphenol (→)-epigallocatechin-3-gallate through autoxidation. *Free Radic Biol Med*. 2008;45(10):1384-1394. doi:10.1016/j.freeradbiomed.2008.07.023

32. Du WJ, Guo JJ, Gao MT, et al. Brazilin inhibits amyloid β-protein fibrillogenesis, remodels amyloid fibrils and reduces amyloid cytotoxicity. *Sci Rep*. 2015;5:7992-8002. doi:10.1038/srep07992

33. Krotee P, Grinder SL, Sawaya MR, et al. Common fibrillar spines of amyloid-β and human islet amyloid polypeptide revealed by microelectron diffraction and structure-based inhibitors. *J Biol Chem*. 2018;293(8):2888-2902. doi:10.7554/eLife.46924

34. Yang F, Lim GP, Begum AN, et al. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J Biol Chem*. 2005;280(7):5892-5901. doi:10.1074/jbc.M404751200

35. Feng Y, Wang XP, Yang SG, et al. Resveratrol inhibits beta-amyloid oligomeric cytotoxicity but does not prevent oligomer formation. *Neurotoxicology*. 2009;30(6):986-995. doi:10.1016/j.neuro.2009.08.013

36. Jimenez-Aliaga K, Bermejo-Bescos P, Benedí J, Martin-Aragon S. Quercetin and rutin exhibit antiamyloidogenic and fibril-disaggregating effects in vitro and potent antioxidant activity in APPswe cells. *Life Sci*. 2011;89(25-26):939-945. doi:10.1016/j.lfs.2011.09.023

37. Durairaj SS, Yuan Q, Xie I, et al. Salvianolic acid B inhibits Abeta fibril formation and disaggregates preformed fibrils and protects against Abeta-induced cytotoxicity. *Neurochem Int*. 2008;52(4-5):741-750. doi:10.1016/j.neuint.2007.09.006

38. Ono K, Yoshiike Y, Takashima A, Hasegawa K, Naiki H, Yamada M. Potent antiamyloidogenic and fibril-destabilizing effects of polyphenols in vitro; implications for the prevention and therapeutics of Alzheimer’s disease. *J Neurochem*. 2003;87(1):172-181. doi:10.1046/j.1471-4159.2003.01976.x

39. Wang Q, Yu X, Patal K, et al. Tanshinones inhibit amyloid aggregation by amyloid-beta peptide, disaggregate amyloid fibrils, and protect cultured cells. *ACS Chem Neurosci*. 2013;4(6):1004-1015. doi:10.1021/cn400051c

40. Li J, Zhu M, Manning-Bog AB, Di Monte DA, Fink AL. Dopamine and L-dopa disaggregate amyloid fibrils: implications for Parkinson’s and Alzheimer’s disease. *FASEB J*. 2004;18(9):962-964. doi:10.1096/fj.03-770fe

41. Luhrs T, Ritter C, Adrian M, et al. 3D of Alzheimer’s amyloid-beta(1-42) fibrils. *Proc Natl Acad Sci U S A*. 2005;102(48):17342-17347. doi:10.1073/pnas.0506723102

42. Iadanza MG, Jackson MP, Hewitt EW, Ranson NA SER. A new era for understanding amyloid structures and disease. *Nat Rev Mol Cell Biol*. 2018;19(12):755-773. doi:10.1038/s41580-018-0060-8

43. Troxt O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multitreading. *J Comput Chem*. 2010;31(2):455-461. doi:10.1002/jcc.21334

44. Windsor PK, Plassmeyer SP, Mattock DS, et al. Bitflavonoid-induced disruption of hydrogen bonds leads to amyloid-β disaggregation. *Int J Mol Sci*. 2021;22(6):2888. doi:10.3390/ijms22062888