SUPPORTING INFORMATION

Vescalagin and castalagin reduce the toxicity of amyloid-beta42 oligomers through the remodelling of its secondary structure

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Note added after first publication: This supplementary information file replaces that originally published on 10 Feb 2020, in which the chemical characterization of the polyphenols vescalagin (1) and castalagin (2) was not clear. Consequently, updated HPLC, MS and \textsuperscript{1}H NMR data are included in this revised version of the SI file (Figures S2-S7), clearly confirming the identity of vescalagin (1) and castalagin (2).

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S1. Materials and methods

1. Purification and characterization of vescalagin (1) and castalagin (2)

The extraction, purification and identification of vescalagin (1) and castalagin (2) was optimized and performed following a previous work.\textsuperscript{1} Briefly, 1 and 2 were obtained from a raw extract from cork powder (Amorim Cork Composites, Portugal) collected by contact with water under reflux for 6h. After cooling, the liquid fraction was filtered and the solvent was partially removed by vacuum evaporation. The final solid extracts were recovered by freeze-drying. The cork water extract was loaded into a semi-preparative chromatographic column, Waters Atlantis OBD Prep T3 (5\textmu m 19x250mm) and 1 and 2 were collected at their respective retention times using the mobile phases A - water:acetic acid 98:2 (v/v) and B - water:acetonitrile:acetic acid 78:20:2 (v/v/v), under the following gradient: 100% A (t=0min) – 100% A (t=15min) – 70% A : 30% B (t=30min) – 100% B (t=35min) – 100% B (t=50min) – 100% A (t=52min) – 100% A (t=57min).

The flow rate was maintained at 5mL/min\textsuperscript{1} and the injection volume was 5mL (Fig. S1). The purity of 1 and 2 was evaluated by HPLC (Waters Acquity, UK) using a 4.6 mm x 250 mm reverse-phase C18 Atlantis column (T3, 5\textmu m, Waters, UK), under a flow of 1 mL/min (injection volume of 20\mu L), using water (2% acetic acid, A) and a mixture of acetonitrile:water:acetic acid (78:20:2, B) as mobile phases, under the following gradient profile: 100% A (t=0min) – 100% A (t=15min) – 70% A : 30% B (t=30min) – 100% B (t=35min) – 100% B (t=50min) – 100% A (t=52min) – 100% A (t=57min). Chromatograms were plotted using the UV detection signal at a wavelength of 280nm (Figs S2-S3). Mass spectra were acquired on an electrospray ionization
(ESI) mass spectrometer (MS) Water Micromass Quattro (Waters, USA) under positive-ion mode (Figs. S4-S5). $^1$H NMR spectra of both 1 and 2 were recorded on a Bruker Avance III spectrometer (Bruker, Germany) at 25°C in a mixture of Acetone-$d_6$:D$_2$O, using a ratio of, approximately, 9:1 (Figs. S6-S7).

2. Peptide preparation

Human amyloid β-peptide (1-42) was obtained by custom synthesis from GeneCust® Europe (Dudelange, Luxembourg). Stock solutions of 0.45mg were prepared by dissolving 10mg of amyloid β-peptide (1-42, Aβ42) in 2.2 mL of HFIP (Fluorochem Ltd, UK) according to the protocol described by Stine et al.$^2$. Briefly, Aβ42 was dissolved in HFIP (5mg/mL) during 30min at room temperature. HFIP was allowed to evaporate in open tubes overnight in the fume hood, and afterwards during an additional 1h under vacuum. A solution of Aβ42 (5mM) in DMSO (i.e. 20µl of fresh dry DMSO to 0.45mg of Aβ42) was sonicated for 10min in an ultra-sound bath. Immediately afterwards, ice-cold water (monomeric form) or 10mM HCl (fibrillar form) was added to a final concentration of 100µM of Aβ42. Finally, the Aβ42 solution was vortex for 15s prior to use.

3. Aβ42 peptide aggregation studies

Thioflavin T (ThT). Fibril formation was followed by ThT assay (assembly, Figs. 2A and S13) during 196h. Aβ42 peptide stock solution was prepared as described above, and fibril formation was induced (under a cold-water bath) by fast mixing of 2µL of Aβ42 DMSO with 98µL of Phosphate Buffer (5mM, with 0.1% of sodium azide, pH 7.2). ThT fluorescence was measured by mixing Aβ42 solution (final concentration of 25µM) with ThT (final concentration of 40µM) and different concentrations of 1 and 2, e.g. concentration ratios Aβ42:polyphenol of 1:0.5; 1:1 and 1:2. The ThT fluorescence was then recorded in a Fluorescence Spectrometer (Jasco, FP-8500, Japan) during 196h using an excitation wavelength of 435nm and an emission wavelength of 465nm. Each experiment was repeated in triplicate. The experiments for the disassembly of the Aβ42 fibrils (Figs. 2B and S14) were performed with Aβ42 fibrillar form, for 24h using the same experimental protocol.

Western Blot (WB). WB analysis of the Aβ42 aggregated forms in the presence and absence of 1 and 2 (at different Aβ42:polyphenol ratios of 1:0.5; 1:1 and 1:2 for 24h or 7 days) allowed the visualization of the relative amount of remodelled peptide. Samples were dissolved on Laemmli buffer (1x) without reducing agent (10µg Aβ42 per lane). Afterwards, samples were electrophoretically resolved in a 12% Bis-Tris Gel Invitrogen NuPAGE, with MES SDS Running Buffer and were transferred to nitrocellulose membranes using iBlot 2 System and blocked with 4% bovine serum albumin (BSA) in TBS containing 0.1% Tween-20 (TBS-T). The membranes were then incubated at 4°C with the 6E10 (anti-Aβ 1-16 antibody – 1:1000) overnight, followed by IRDye 800CW Goat anti-Mouse IgG Secondary Antibody (RT, during 1.5h;1:10000). After each antibody incubation, the membranes were washed with TBS-T. Signal were detected (acquisition time: 2min) in Odyssey Fc Imaging System (LI-COR Inc., Nebraska USA).

Circular Dichroism (CD). CD was performed using a 1mm path length cell at 37°C in a CD spectrometer (Jasco, J1500, Japan). Spectra (Figs. S20-S21) were recorded in the range between 190–260nm with a scan rate of 10nm/min and a response time of 1s. Three scans were accumulated for each spectrum. For all the CD experiments, the Aβ42 concentration was 25µM, and the Aβ42/polyphenol ratios were 1:0.5 and 1:1. Results are expressed as $\theta$ [mdeg].
Isothermal Titration Calorimetry (ITC). ITC was used to evaluate the interactions between 1 and 2 and Aβ42. ITC measurements were performed using a MicroCal VP-ITC (MicroCal Inc., Northampton, MA, USA). Samples were degassed in a ThermoVac system (MicroCal) prior to use. 1 (or 2) was titrated into an Aβ42 solution (10µM) in PBS. A first injection of 2µL (neglected in the analysis) followed by other 27 injections of 10µL each were performed under continuous stirring at 286 rpm. All the measurements were done at 25°C. PBS buffer was titrated to peptide solution to establish the baseline analysis. ITC experiment offers the basic thermodynamic profile for the established interactions, including three key binding parameters: Gibbs energy (that can be calculated from the equilibrium association constant K), enthalpy and entropy of interaction:

$$\Delta G = -RT \ln K = RT \ln kd$$

where K is the equilibrium association constant, kd is the equilibrium dissociation constant, T is the thermodynamic or absolute temperature and R is the gas constant.

The binding Gibbs energy change can be calculated from the enthalpic and entropic contributions by means of:

$$\Delta G = \Delta H - T\Delta S$$

to obtain such thermodynamic parameters, the raw data of ligand interaction were analysed by fitting the heat isotherms by a nonlinear least-squares analysis to a one-binding-site model.

Atomic Force Microscopy (AFM). For the acquisition of AFM images (Figs. 3B, S22-S23), freshly cleaved mica was functionalized with a drop of (3-Aminopropyl)triethoxysilane (APTES, 200μL), during 30 min at room temperature. Then, micas were rinsed with deionized water and dried under a nitrogen flux. Each sample, Aβ42 peptide (10µM) in the presence and absence of 1 and 2, were spotted onto the functionalized mica during 30min, and then washed with water and dried under nitrogen.

AFM images were acquired using a JPK Nanowizard 3 (JPK, Germany) in air at room temperature under AC mode. The scans were acquired at a 512 x 512 pixels resolution using ACTA-SS probes (k∼37N/m, AppNano, USA), a drive frequency of ∼254kHz, a setpoint of ∼0.5V and a scanning speed of 1.0Hz.

4. Cell toxicity assays

Neuroblastoma SH-SY5Y cells were cultured at 37°C in a humidified 95/5% air/CO₂ atmosphere using Dulbecco's modified Eagles medium F-12 (Gibco, UK) supplemented with 10% FBS (Gibco, UK) and 1% ATB (Gibco, UK) solution. Cell medium were replaced each 2 days and cells were sub-cultured once they reached 90% confluence. Cells were plated at a density of 25 000 cells per well on 96-well plates containing DMEM/F-12 media (for MTS assay) and plated at a density of 50 000 cells per well on 24-well plates containing DMEM/F-12 media (for the live/dead assay). A typical experiment included the culture of the neuroblastoma cell line (SHSY-5Y) during 24h in the absence or presence of 1 or 2 at different concentrations. Afterwards, Aβ42 were added to the culture medium, and, after an additional 24h, the cells were evaluated for their metabolic activity. Both 1 and 2 were sterilized by autoclaving before use. Aβ42 peptide was sterilized by UV and immediately added to the cells after being reconstituted in DMSO (0.02%) and diluted into DMEM/F-12 media.
MTS assay. The Aβ42 cytotoxicity (Figs. S25-S26 and S29) was measured using a colorimetric assay for assessing cell metabolic activity, 24h after the addition of Aβ42 at a concentration of 25µM. The absorbance of the metabolic activity was assessed by MTS (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega) according to the supplier’s instructions. The relative metabolic activity (%) of the SH-SY5Y cells was determined for each experimental condition. The optical density (OD) was recorded at 490nm with a Synergy HT microplate reader (Bio-Tek Instruments). p-values were calculated using two-tailed t-test. Results are presented as mean ± SEM of 6 independent experiments for each experimental condition.

Live/Dead assay. Cell viability was also evaluated by Live/Dead assay using calcein AM to stain live cells and propidium iodide (PI) to stain dead cells (Figs. 4A and S27). Viable cells were stained in green and dead cells were stained in red. Shortly, cells were incubated for 20min with both dyes and then observed under a fluorescence microscope (Axio Imager Z1m, Zeiss).

Protein expression. For immunostaining, fluorescence images (Figs. 4B and S30), after 24h of culture, the samples were washed twice with PBS, fixed in 10% neutral buffered formalin for 30min at 4°C, permeabilised with 0.1% Triton X-100 in PBS for 5min, and blocked with 3% BSA in PBS for 30min at room temperature. To evaluate the accumulation of Aβ42 in its different forms, a primary antibody against Aβ42 (1-16) (Biotin anti-β-Amyloid, I-16 Antibody, Mouse IgG1 1:200 in 1% w/v BSA/PBS, Biolegend) was employed, followed by rabbit anti-mouse Alexafluor-488 (1:500 in 1% w/v BSA/PBS, anti-mouse, Invitrogen). A phalloidin–TRITC conjugate was used (1:200 in PBS for 30min, Sigma) to assess cytoskeleton organisation. Nuclei were counterstained with 1mg/mL of 4,6-diamidino-2-phenylindole (DAPI; Sigma) for 30min. Samples were washed with PBS, mounted with Vectashield® (Vector) on glass slides and observed under a confocal laser scanning microscope (Leica TCS SP8, Leica Microsystems).

S2. Purification and characterization of vescalagin (1) and castalagin (2)

Fig. S1 A. Preparative HLPC chromatogram of cork water extract with the identification of the peaks that correspond to vescalagin (1) and castalagin (2). B. Chemical structure of both isomers, vescalagin (1) and castalagin (2), with the position C1 (that identifies the main structural difference between 1 and 2) highlighted.
HPLC characterization of purified 1 and 2.

Fig. S2 HPLC run of vescalagin (1) purified from cork water extract.

Fig. S3 HPLC run of castalagin (2) purified from cork water extract.
ESI-MS (m/z): [M+H]+ 935

Fig. S4 Positive ESI-MS spectrum of vescalagin (1).

Fig. S5 Positive ESI-MS spectrum of castalagin (2).
\(^1\)H NMR (400 MHz, D\(_2\)O, 298 K)

\(^1\)H NMR spectra of both vescalagin (1) and castalagin (2) was recorded on a Bruker Avance III spectrometer (Bruker, Germany) at 25 °C in Acetone-d\(_6\):D\(_2\)O, using a ratio of, approximately, 9:1 (Figs. S6-S7). The assignments reported below were made following the ones reported by Puech et al.\(^3\) and Douat et al.\(^4\)

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**Fig. S6** \(^1\)H NMR spectrum of vescalagin (1) isolated from cork water extract. **A.** Chemical shift range between 0-9 ppm; **B.** Zoom of the chemical shift range between 3.5-7.5 ppm showing the most representative vescalagin (1) peaks.
Fig. S7 $^1$H NMR spectra of castalagin (2) isolated from cork water extract. A. Chemical shift range between 0-9ppm;
B. Zoom of the chemical shift range between 3.5-7.5 ppm showing the most representative castalagin (2) peaks.
Vescalagin (1)

$^1$H NMR (400 MHz, Acetone-$d_6$:D$_2$O – 9:1 ratio, 298 K): δ 6.76 (s, 1H); δ 6.74 (s, 1H); δ 6.59 (s, 1H); δ 5.59 (d, $J = 7.9$ Hz, 1H); δ 5.23 (t, $J = 1.9$ Hz, 1H); δ 5.16 (t, $J = 7.2$ Hz, 1H); δ 5.02 (dd, $J = 13.0$ Hz, 2.7 Hz, 1H); δ 4.85 (d, $J = 2.1$ Hz, 1H); δ 4.52 (dd, $J = 7.0$ Hz, 1.5 Hz, 1H); δ 4.00 (d, $J = 12.9$ Hz, 1H).

Castalagin (2)

$^1$H NMR (400 MHz, Acetone-$d_6$:D$_2$O – 9:1 ratio, 298 K): δ 6.78 (s, 1H); δ 6.76 (s, 1H); δ 6.62 (s, 1H); δ 5.67 (d, $J = 4.6$ Hz, 1H); δ 5.56 (d, $J = 7.1$ Hz, 1H); δ 5.20 (t, $J = 7.2$ Hz, 1H); δ 5.09-4.95 (m, 3H); δ 4.00 (d, $J = 12.9$ Hz, 1H).

Fig. S8 A. Fluorescence spectrum of vescalagin (1, 50 μM) acquired using a $\lambda_{ex}$ = 435 nm and a $\lambda_{em}$ = 445-600 nm; B. 3D fluorescence spectra for vescalagin (1, 50 μM) using the following acquisition parameters: $\lambda_{ex}$ = 300-500 nm; $\lambda_{em}$ = 300-600 nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.

Fig. S9 A. Fluorescence spectrum of castalagin (2, 50 μM) acquired using a $\lambda_{ex}$ = 435 nm and a $\lambda_{em}$ = 445-600 nm; B. 3D fluorescence spectra for castalagin (2, 50 μM) using the following acquisition parameters: $\lambda_{ex}$ = 300-500 nm; $\lambda_{em}$ = 300-600 nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.
S3. Aβ42 peptide aggregation studies

Fig. S10 HPLC run of the Aβ42 peptide used throughout the present work. A sample of peptide was dissolved in acetonitrile (0.02% DMSO) and the HPLC run was performed using a reverse phase C18 column (4.6x250mm, Waters, UK), a mixture of eluents (A – acetonitrile, 0.1% trifluoroacetic acid, and B – water, 0.1% trifluoroacetic acid), starting with 10% A (t=0min) and ending with 100% B (t=20min); using a flow rate of 1.0mL/min; column.

Fig. S11 Positive ESI-MS spectrum of Aβ42 sample used throughout the present work, showing the characteristic [M+5H]^{5+} and [M+6H]^{6+} peaks of Aβ42.
Fig. S12 Aβ42 aggregation kinetics monitored by the ThT binding assay. Aβ42 samples were prepared by two methods: Method 1 (red line) - Aβ42 was dissolved in 10% (w/v) NH₄OH at a concentration of 0.5mg/ml. The peptide was incubated for 10min at room temperature followed by sonication (5min). The NH₄OH was removed by lyophilization overnight. Immediately prior to use, the Aβ42 was dissolved in 60mM NaOH; Method 2 (blue line) - Aβ42 was dissolved in HFIP (5mg/ml) at room temperature, during 30min. We allowed HFIP to evaporate in open tubes overnight in the fume hood, and then during 1h under vacuum. An aliquot of 5mM Aβ42 in DMSO (20μl of fresh dry DMSO to 0.45mg of Aβ42) was sonicated for 10min in a bath sonicator. Immediately after, ice-cold water was added to a final concentration of 100μM of Aβ42, followed by vortexing during 15s. ThT fluorescence was monitored in 0.1mM phosphate buffer, pH 7.2, 0.02% NaN₃ (using 25μM of Aβ42).

Fig. S13 Aβ42 assembly kinetics (Method 2) using the ThT assay. A. Assembly of Aβ42 in the presence of different concentrations of 1. B. Assembly of Aβ42 in the presence of different concentrations of 2. Compounds were added at the lag phase of the Aβ42 aggregation profile and fluorescence was measured over 140h. Both experiments (A-B) were performed at room temperature. ThT fluorescence data collected using the following parameters: λₑₓ = 435nm, λₑₓₑ = 465nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.
Fig. S14 Aβ42 disassembly kinetics (Method 2) using the ThT assay. **A.** Disassembly of Aβ42 in the presence of different Aβ42:polyphenol ratios for 1. **B.** Disassembly of Aβ42 in the presence of different Aβ42:polyphenol ratios for 2. Compounds were added at the plateau phase of the Aβ42 aggregation profile and fluorescence was measured over 24h. Both experiments (A-B) were performed at room temperature. ThT fluorescence data collected using the following parameters: λ_ex = 435nm, λ_em = 465nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.

Fig. S15 **A.** Representative WB image and **B.** relative densitometric bar graphs of the Aβ42 assembly in the presence of vescalagin (1) and castalagin (2). Both compounds were incubated during 24h (under Aβ42:polyphenol ratios of 1:0.5, 1:1 and 1:2). WBs were performed in a NuPAGE 12% Bis-Tris Gels, using MES running buffer, and incubated with 6E10 (anti-Aβ1-16) antibody.
**Fig. S16 A.** Representative WB image and B. relative densitometric bar graphs of the Aβ42 assembly in the presence of vescalagin (1) and castalagin (2). Both compounds were incubated during 7 days (under Aβ42:polyphenol ratios of 1:0.5, 1:1 and 1:2). WBs were performed in a NuPAGE 12% Bis-Tris Gels, using MES running buffer, and incubated with 6E10 (anti-Aβ1-16) antibody.

**Fig. S17 A.** Representative WB image and B. relative densitometric bar graphs of the Aβ42 disassembly in the presence of vescalagin (1) and castalagin (2). Both compounds were incubated during 24h (under Aβ42:polyphenol ratios of 1:0.5, 1:1 and 1:2). WBs were performed in a NuPAGE 12% Bis-Tris Gels, using MES running buffer, and incubated with 6E10 (anti-Aβ1-16) antibody.
**Fig. S18** A. Representative WB image and B. relative densitometric bar graphs of the Aβ42 disassembly in the presence of vescalagin (1) and castalagin (2). Both compounds were incubated during 7 days (under Aβ42:polyphenol ratios of 1:0.5, 1:1 and 1:2). WBs were performed in a NuPAGE 12% Bis-Tris Gels, using MES running buffer, and incubated with 6E10 (anti-Aβ1-16) antibody.

**Fig. S19** Relative densitometric bar graphs of Aβ42 assembly and disassembly for 24h and 7 days, respectively. WBs were performed in a NuPAGE 12% Bis-Tris Gels, using MES running buffer, and incubated with 6E10 (anti-Aβ1-16) antibody.
Fig. S20 CD spectra of Aβ42 peptide (25μM) in the presence and absence of vescalagin (1) or castalagin (2) during 7 days. Amyloid-aggregates have a β-sheet-rich secondary structure after 7 days. In all the cases, incubation was made at 37°C, under constant agitation. The interaction between the Aβ42 and 1 or 2 results in a blue shift in the characteristic curves of the β-sheet-rich structures of Aβ42, especially for the one at ~220nm.

Table S1 Secondary structure of Aβ42 during assembly (aggregation from the monomeric form) and disassembly (disaggregation of the pre-formed fibrils) in the presence of vescalagin (1) and castalagin (2). Values are in percentage obtained by fitting the CD spectra using the BeStSel method. Parallel β-sheets are usually reported to be the major structure present in Aβ42 fibrils, while antiparallel β-sheets are usually assigned to on- and off-pathway Aβ42 oligomeric structures (including cytotoxic and non-cytotoxic forms).

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<th>Secondary structure</th>
<th>Aβ42 (Day 0)</th>
<th>Aβ42 (Day 7)</th>
<th>Aβ42 + 1 (Day 7)</th>
<th>Aβ42 + 2 (Day 7)</th>
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<th>Aβ42 (Day 1)</th>
<th>Aβ42 + 1 (Day 1)</th>
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Fig. S21 CD spectra of Aβ42 pre-formed fibrils in the absence and presence of vescalagin (1) or castalagin (2) during 1 day. In all the cases, incubation was made at 37°C, under constant agitation.

Fig. S22 AFM images of Aβ42 fibrils formed for 10 days (10μM). Vescalagin (1) and castalagin (2) were added into an Aβ42 solution (ratios Aβ42:polyphenol of 1:1 and 1:2) and left to incubate for 24h under constant agitation. Both compounds directly modified Aβ42 fibrils and oligomers (green arrows: aggregates with ≈30 nm; blue arrows: ≈70 nm); scale bars 2 μm and 200 nm.
Fig. S23 AFM representative images of Aβ42 (25μM). A. Assembly of Aβ42: vescalagin (1) and castalagi (2) were added into an Aβ42 monomeric solution (ratio of Aβ42:polyphenol of: 1:1) and left to incubate for 7 days; B. Disassembly of Aβ42: vescalagin (1) and castalagi (2) were added into a solution of Aβ42 pre-formed fibrils (ratio of Aβ42:polyphenol of: 1:1) and left to incubate for 7 days. Scale bar = 1 μm and 500 nm. Green arrows: aggregates generated in the presence of vescalagin (1) with ≈50nm (assembly) and ≈200nm (disassembly). Blue arrows: aggregates generated in the presence of castalagi (2) with ≈80nm (assembly) and ≈100nm (disassembly). Scale bars = 2 μm and 200 nm.

Fig. S24 Isothermal titration calorimetry (ITC) curves for the binding of vescalagin (1) and castalagi (2) to Aβ42 peptide: $K_1$ (mol$^{-1}$)= 1.26E6±0.18E6; $K_2$ (mol$^{-1}$)= 4.54E6±0.56E6.
S4. Cell toxicity assays

Fig. S25 SH-SY5Y cell viability in the presence of Aβ42 fibrils during 24h. Cells were incubated during 24h with different concentrations of freshly prepared Aβ42. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (vs. control); n=3.

Fig. S26 MTS assay: SH-SY5Y metabolic activity in the presence of a solution of Aβ42 pre-formed fibrils (25µM) and filtered Aβ42 (25µM) during 24h. Fibrillar Aβ42 was filtered with a 0.22µm filter. *** $p < 0.001$ (vs cells: without Aβ42) n=3; # $p < 0.05$ (vs fibrillar Aβ42); n=3.
Fig. S27 Fluorescence quantification of live cells stained in green (calcein) in the presence of a solution of Aβ42 pre-formed fibrils (25µM) and filtered fibrillar Aβ42 (25µM), during 24h. Fibrillar Aβ42 was filtered with a 0.22µm filter. *** $p < 0.001$ (vs cells: without Aβ42); n=3; * $p < 0.05$ (vs fibrillar Aβ42); n=3. B. Representative images of the Live/dead assay. Scale bar = 50 µm.

Fig. S28 Live/Dead assay of SH-SY5Y: A. fluorescence quantification of live cells stained in green (calcein) in the presence of a solution of Aβ42 pre-formed fibrils (25µM) and vescalagin (1) or castalagin (2) at different molar ratios (Aβ42:polyphenol 1:0.5; 1:1; 1:2) during 24h. *** $p < 0.001$ (vs control); ### $p < 0.001$ (vs 25µM Aβ42); n=3.
**Fig. S29** A. MTS assay: SH-SY5Y cell viability in the presence of Aβ42 (25µM) and vescalagin (1) or castalagin (2) at different molar ratios (Aβ42:polyphenol 1:0.5; 1:1; 1:2) during 24h. * p < 0.05, ** p < 0.01, *** p < 0.001 (vs control); n=4. B. Fluorescence intensity of the Aβ42 immunostaining (6E10 antibody) after SH-SY5Y cell culture in the absence and presence of vescalagin (1) and castalagin (2), at different Aβ42:polyphenol ratios, 1:0.5, 1:1 and 1:2, for 24h. Fluorescence was quantified using the image processing package Fiji (http://fiji.sc/wiki/index.php/Fiji).

**Fig. S30** Representative fluorescence microscopy images of SH-SY5Y cells treated with different molar ratios of Aβ42:polyphenol, *i.e.* 1:0.5, 1:1 and 1:2, of vescalagin (1) and castalagin (2), as well as Aβ42 (25µM) during 24h. Immunostaining of Aβ42 (6E10, Aβ1-16) (green), actin (red) and nuclei (blue). Scale bar = 50 µm.

**References:**