Title: Yet another "*in vitro*" evidence that natural compounds introduced by diet have antiamyloidogenic activities and can counteract neurodegenerative disease depending on aging

Anna Lia Asti[1], Stefania Crespi[2], Teresa Rampino[1], Paola Zelini[3], Marilena Gregorini[1,8], Alessia Pascale[4], Nicoletta Marchesi[4], Stefania Saccucci[5], Carla Colombani[6], Sara Vitalini[7] ,Marcello Iriti[7]

[1]*Unit of Nephrology, Dialysis and Transplantation, Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia, Italy;*
[2] *Department of Earth Sciences Ardito Desio, University of Milan, Milan, Italy*
[3]*Unit of Obstetrics and Gynecology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;*
[4]*Department of Drug Sciences, Pharmacology Section, University of Pavia, Pavia, Italy*
[5] *Unitech NoLimi, University of Milan, Milan ,Italy*
[6]*Department of Agricultural and Environmental Sciences Territorial Production and Agroenergy, University of Milan, Milan, Italy*
[7]*Department of Biomedical, Surgical and Dental Sciences, University of Milan,, Milan,, Italy* [8]
Department of Internal Medicine and Therapeutics, University of Pavia, 27100 Pavia, Italy.

Corrisponding author: Anna Lia Asti
annalia.asti@unipv.it mobile+39
3333962590

Abstract

A major issue in Alzheimer's disease (AD) research is to find some new therapeutic drug which decrease Amyloid-beta (Aβ) aggregation. From a therapeutic point of view the major question is whether pharmacological inhibition of inflammation pathways will be able to safely reverse or slow the course of disease. Natural compounds are capable of binding to different targets implicated in AD and exert neuroprotective effects. Aim of this study was to evaluate the *in vitro* inhibition of $A\beta_{1-42}$ fibrillogenesis in presence of Gallic acid, Rutin, Melatonin and Provinols™ . We performed the analysis with Transmission and Scanning Electron Microscopy, and with X-ray microanalysis. Samples treated with Rutin, that arises from phenylalanine via the phenyl propanoid pathway, show the best effective result obtained because a significantly fibril inhibition activity is detectable compared to the other compounds. Melatonin shows a better inhibitory activity than Provinols™ and Gallic acid at the considered concentrations.

3. Experimental

Aβ1-42 fragment

Aβ1-42 fragment (SIGMA-Aldrich Chemie, Germany) was dissolved in DMSO and then diluted in PBS, to obtain a final concentration of 0,5 μg/ml and observed by TEM after three days incubation at 37◦C. Different aliquots of Aβ1-42 fibrils were processed with different concentrations of Rutin, Provinols™, Gallic acid and Melatonin for 24 hrs at 37° and observed by TEM.

Congo Red (CR)

Amyloid stained with CR gives an apple-green birefringence when viewed at polarized light.

Substances with anti-amyloidogenic activity

All the compounds was dissolved in DMSO and then diluited with PBS: Rutin, PM=610,517 g/mol at the concentration of 100 and 200μM; Gallic acid PM= 170,12 g/mol at the concentration of 100 and 200μM, Melatonin, PM= 232,278 g/mol at the concentration of 50 and 100 μM. All the compounds were incubated with Aβ1-42 for 24 hrs. Provinols™ was prepared at the same concentration of Aβ1-42 fragment. The compounds were purchased from SIGMA-Aldrich Chemie, Germany.

Transmission electron microscopy (TEM)

Samples were prepared with the Negative Staining technique by floating small aliquots (20μl) of aqueous suspension on formvar/carbon coated glow-discharged grids for 2 minutes; then air dried and stained with 2% uranyl acetate for 2–3 minutes.. The observations and micrographs were performed with a JEOL JEM 1200 EX electron microscope operating at 100 kV.

Energy dispersive X-ray spectroscopy (EDS)

EDS technique, mostly used for qualitative analysis of materials but also providing semi-quantitative results, was performed to identify the elemental composition of the compounds, as well as their quantities. The microanalysis was performed with a JEOL, EDS-SEM JSM-IT500 LV

Backscattered electron (BSE) and Scanning electron microscopy (SEM)

For SEM samples were fixed in glutaraldehyde 2.5% and cacodylate sodium buffer pH 7.4 for 2 hrs at room temperature, then rinsed in cacodylate sodium buffer (pH 7.4) Dehydration was performed at

increasing ethanol concentrations (50% to 100%). Secondary and backscattered electrons are used in image forming for morphological analysis; samples were fixed with a carbon paint on aluminium stubs and covered with a carbon layer with a Sputter Coater Edwards S 150 A. Observations carried out with a scanning electron microscope JEOL, EDS-SEM JSM-IT500 LV .

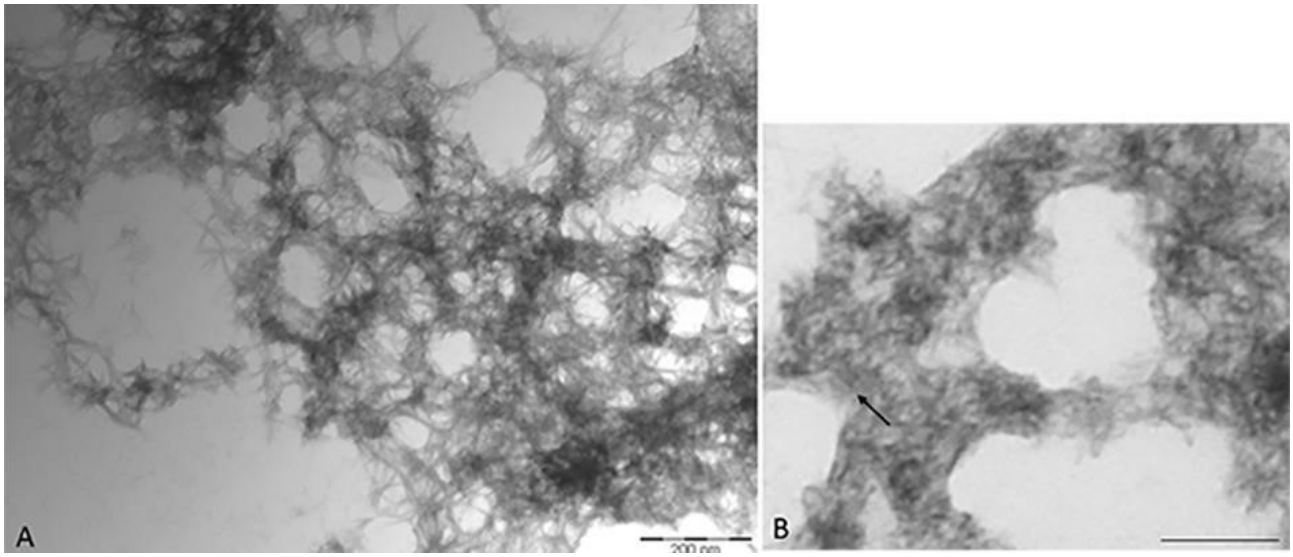
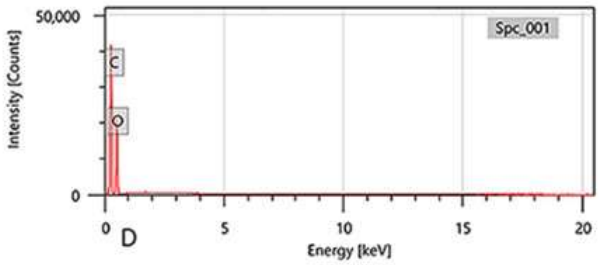
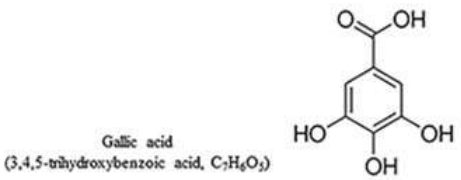


Figure S1



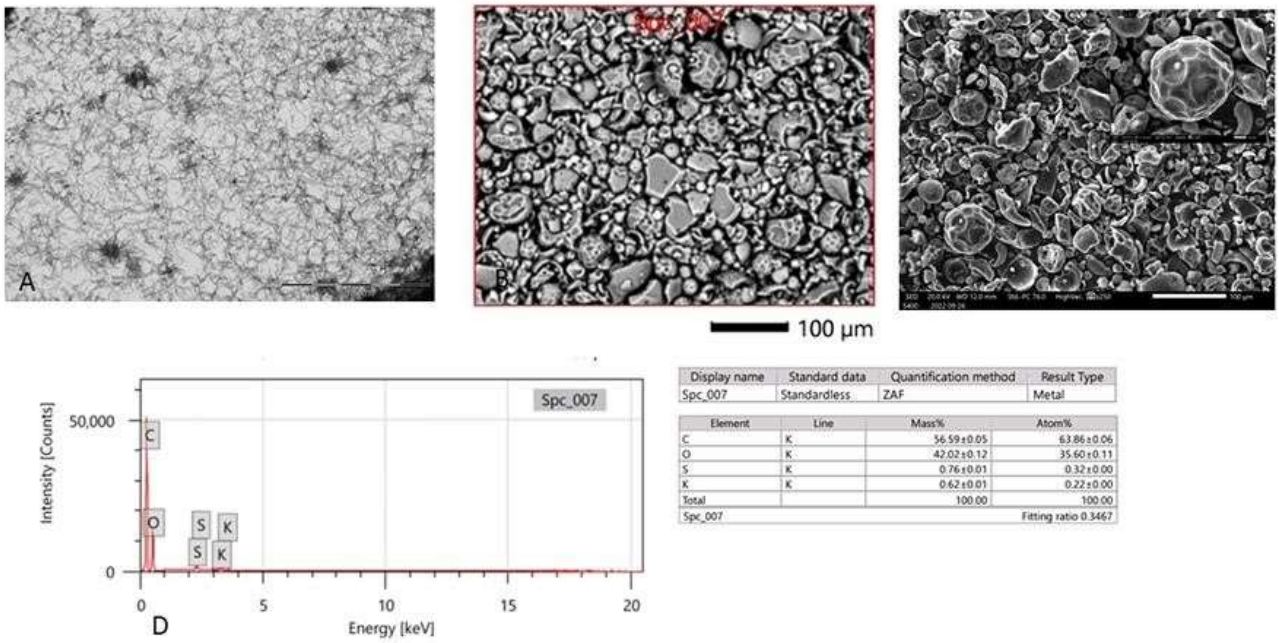
Display name	Standard data	Quantification method	Result Type
Spc_001	Standardless	ZAF	Metal

Element	Line	Mass%	Atom%
C	K	47.93±0.05	55.08±0.05
O	K	52.07±0.13	44.92±0.11
Total		100.00	100.00
Spc_001			Fitting ratio 0.3258



Gallic acid
(3,4,5-trihydroxybenzoic acid, $C_7H_6O_5$)

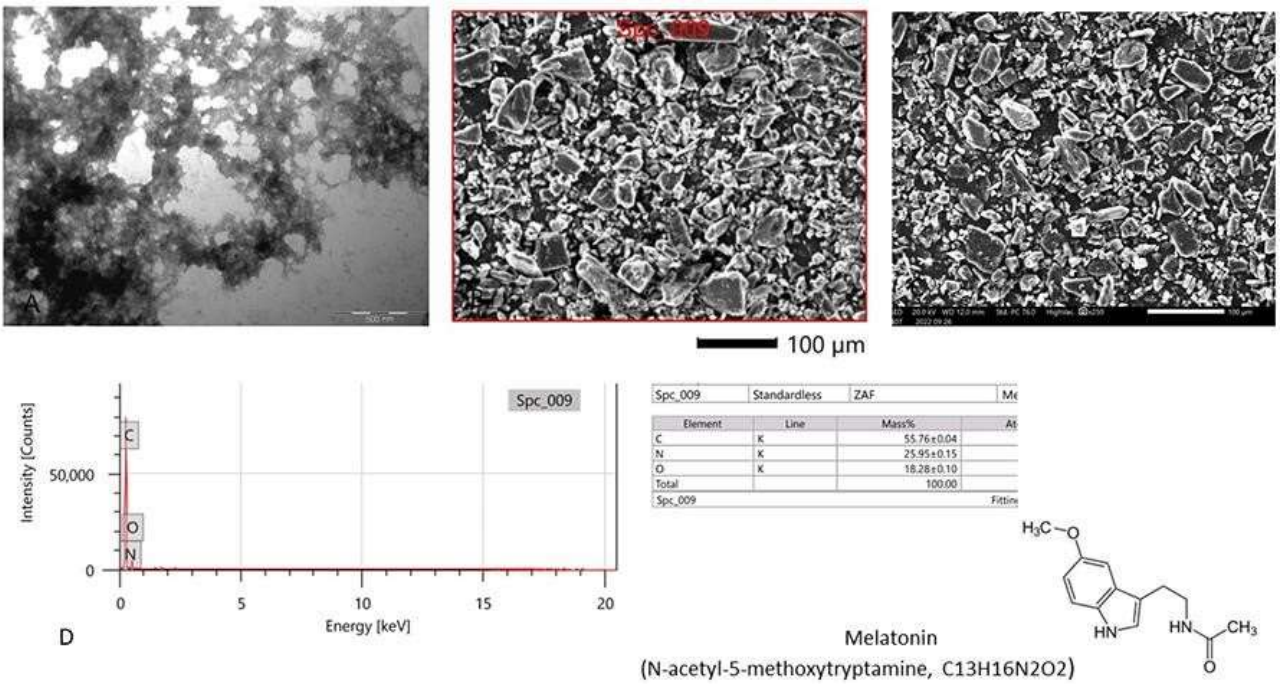
Figure S2



Display name	Standard data	Quantification method	Result Type
Spc_007	Standardless	ZAF	Metal

Element	Line	Mass%	Atom%
C	K	56.59±0.05	63.86±0.06
O	K	42.02±0.12	35.60±0.11
S	K	0.76±0.01	0.32±0.00
K	K	0.62±0.01	0.22±0.00
Total		100.00	100.00
Spc_007			Fitting ratio 0.3467

Figure S3



Spc_009	Standardless	ZAF	Me

Element	Line	Mass%	At
C	K	55.76±0.04	
N	K	25.95±0.15	
O	K	18.28±0.10	
Total		100.00	
Spc_009			Fittin

Figure S4

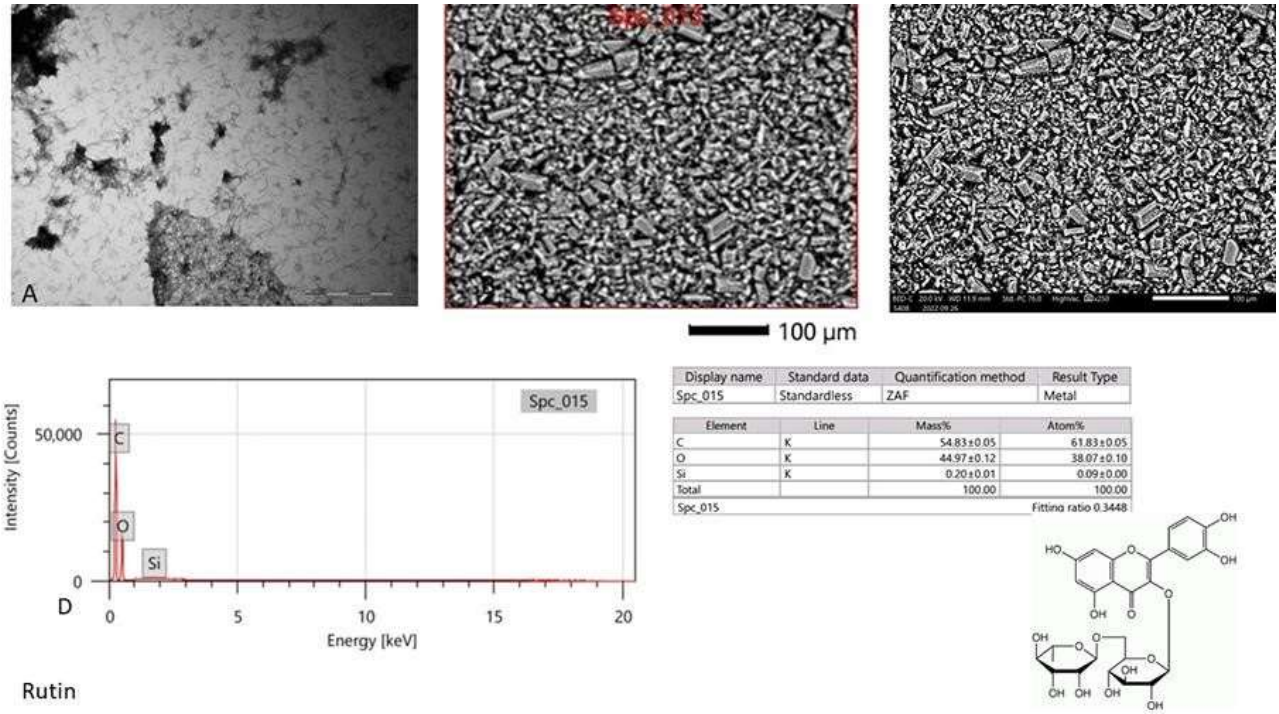


Figure S5

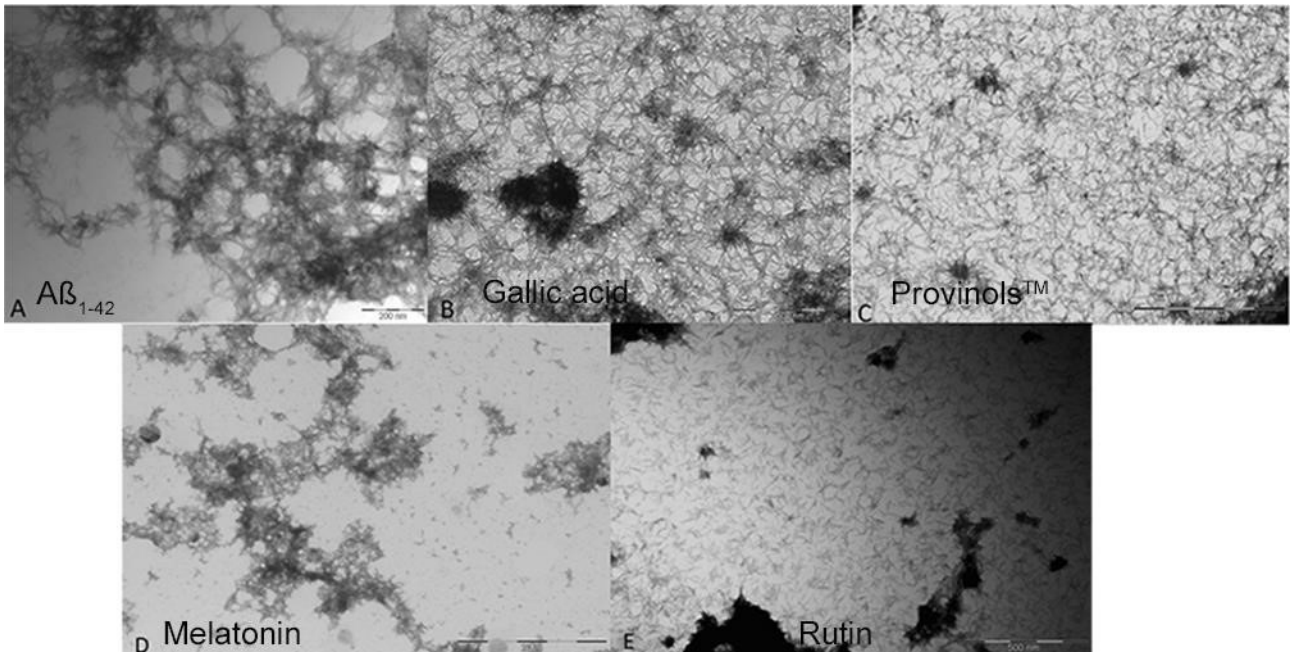


Figure S6

Legends

Figure S1. Spontaneous *in vitro* fibrillogenesis of $A\beta_{1-42}$ peptide. A) long irregular flexous fibrils and regular helical twisted fibrils are visible, bar=200nm. In B) at greater magnification micelles are also detectable,, bar=100nm

Figure S2. *$A\beta_{1-42}$ in presence of Gallic acid,* 200µM A) shorter fibrils appear arranged in a network with different nucleations centers, bar= 500nm. B) SEM of crystalline structure, bar=100µm C) BSE of different phases of Gallic acid; D) elemental composition

Figure S3. *$A\beta_{1-42}$ in presence of Provinols^{TM}* A) fibrils are thinning, frials and less numerous then those observed in Fig 1A, bar= 500nm; B) SEM of the crystalline structure, bar=100µm; C) BSE of different phases of *Provinols^{TM}* ; D) elemental composition

Figure S4. *$A\beta_{1-42}$ in presence of Melatonin,* 100µM. *A)* amorphous material and not clearly identificable fibrils are detectable, bar=500nm; B) SEM of the crystalline structure, bar=100µm C) BSE of different phases Melatonin; D) elemental composition

Figure S5. *Analysis of $A\beta_{1-42}$ in presence of Rutin,* 200µM. *A)* short and not organized fibrils and numerous oligomers are also detectable, bar=500nm; B) SEM of the crystalline structure, bar=100µm C) BSE of different phases of Melatonin; D)) elemental composition

Figure S6. A) *Aβ without compounds*, bar=200nm; B) Aβ in presence of Gallic acid, bar500 nm; C) Aβ in presence of Provinols^{TM}, bar =500nm; D) Aβ in presence of Melatonin, bar=500nm,; E) Aβ in presence of Rutin, bar=500nm