Food Chemistry 199 (2016) 185–194

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Metal chelation, radical scavenging and inhibition of $A\beta_{42}$ fibrillation by food constituents in relation to Alzheimer's disease

Stephen Chan [a,1], Srinivas Kantham [a,1], Venkatesan M. Rao [a], Manoj Kumar Palanivelu [a], Hoang L. Pham [a], P. Nicholas Shaw [a], Ross P. McGeary [b], Benjamin P. Ross [a,*]

[a] The University of Queensland, School of Pharmacy, Brisbane, Queensland 4072, Australia
[b] The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Queensland 4072, Australia

ARTICLE INFO

*Article history:*
Received 30 May 2015
Received in revised form 19 October 2015
Accepted 26 November 2015
Available online 26 November 2015

*Chemical compounds:*
L-Ascorbic acid (PubChem CID: 54670067)
Caffeic acid (PubChem CID: 689043)
Caffeine (PubChem CID: 2519)
Curcumin (PubChem CID: 969516)
(−)-Epigallocatechin gallate (PubChem CID: 65064)
Gallamide (PubChem CID: 69256)
Gallic acid (PubChem CID: 370)
Propyl gallate (PubChem CID: 4947)
Resveratrol (PubChem CID: 445154)
α-Tocopherol (PubChem CID: 14985)

*Keywords:*
Polyphenols
Vitamins
Metal chelators
Antioxidants
Amyloid-beta peptide

ABSTRACT

Various food constituents have been proposed as disease-modifying agents for Alzheimer's disease (AD), due to epidemiological evidence of their beneficial effects, and for their ability to ameliorate factors linked to AD pathogenesis, namely by: chelating iron, copper and zinc; scavenging reactive oxygen species; and suppressing the fibrillation of amyloid-beta peptide (Aβ). In this study, nine different food constituents (L-ascorbic acid, caffeic acid, caffeine, curcumin, (−)-epigallocatechin gallate (EGCG), gallic acid, propyl gallate, resveratrol, and α-tocopherol) were investigated for their effects on the above factors, using metal chelation assays, antioxidant assays, and assays of $A\beta_{42}$ fibrillation. An assay method was developed using 5-Br-PAPS to examine the complexation of Zn(II) and Cu(II). EGCG, gallic acid, and curcumin were identified as a multifunctional compounds, however their poor brain uptake might limit their therapeutic effects. The antioxidants L-ascorbic acid and α-tocopherol, with better brain uptake, deserve further investigation for specifically addressing oxidative stress within the AD brain.



## 1. Introduction

Alzheimer's disease (AD) is the most common form of dementia, and in 2013 there were 44 million people with dementia worldwide, with this number expected to increase to 76 million by 2030, and 135 million by 2050 (Prince, Guerchet, & Prina, 2013). AD involves a gradual worsening in memory and cognitive function, deficits that can be improved modestly with the use of symptomatic drugs, such as acetylcholinesterase inhibitors and *N*-methyl-D-aspartate receptor antagonists (Anand, Gill, & Mahdi, 2014). However, there is currently no disease-modifying treatment that can halt or slow the progression of AD. Various foods and their chemical constituents have been proposed as disease-modifying agents for AD, supported by epidemiological evidence of their beneficial effects. For example, better cognitive function has been observed in older populations that have a high intake of curries (Ng et al., 2006) and teas (Ng, Feng, Niti, Kua, & Yap, 2008). This phenomenon has been attributed to the medicinal properties of curcumin, which is found in the spice turmeric (Ringman, Frautschy, Cole, Masterman, & Cummings, 2005) that is an ingredient of curries, as well as (−)-epigallocatechin gallate (EGCG) and gallic acid which are found in teas (Bastianetto, Yao, Papadopoulos, & Quirion, 2006). It has been reported that higher consumption of coffee in mid-life is associated with a lower risk of dementia and AD in the later years of life (Eskelinen, Ngandu, Tuomilehto, Soininen, & Kivipelto, 2009). A lower dementia risk

* Corresponding author.
*E-mail address:* b.ross1@uq.edu.au (B.P. Ross).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2015.11.118
0308-8146/

was also seen in groups consuming wine, but the consumption of spirits increased the risk, suggesting that wine components, such as polyphenols, are responsible for the risk-modifying effect rather than ethanol (Mehlig et al., 2008). Other food constituents, such as L-ascorbic acid found in citrus fruits, and α-tocopherol found in nuts, have been promoted as antioxidants of benefit in AD (Dysken et al., 2014; Harrison, Bowman, & Polidori, 2014). The beneficial effects of the aforementioned foods might be attributed to the ability of their constituents to ameliorate specific factors linked to AD pathogenesis. According to the amyloid cascade hypothesis, amyloid-beta peptide (Aβ) accumulation is the primary event in AD pathogenesis, and soluble Aβ oligomers are the main neurotoxic species due to cell membrane perturbation, oxidative stress, and other mechanisms (Fandrich, 2012). Aβ contains metal binding sites, and metals, such as copper, zinc and iron, are present at elevated concentrations in Aβ plaques (Greenough, Camakaris, & Bush, 2013). Metals can affect the morphology of Aβ aggregates, accelerate Aβ fibrillation, and increase the cytotoxicity of Aβ (Viles, 2012). Redox active copper and iron in complex with Aβ can generate hydrogen peroxide, which may be converted to hydroxyl radicals via Fenton chemistry, resulting in oxidative cellular damage (Greenough et al., 2013). Consequently, food constituents that can suppress Aβ fibrillation, chelate metal ions, and scavenge free radicals, may be of benefit in AD.

In this study, nine different food constituents (Fig. 1; L-ascorbic acid, caffeic acid, caffeine, curcumin, EGCG, gallic acid, propyl gallate, resveratrol, and α-tocopherol) were investigated, *in vitro*, for their effects on the above factors using assays that examine metal chelation, antioxidant properties and inhibition of $A\beta_{42}$ fibrillation. An aim of the study was to determine the relative potencies of the food constituents, and to identify multifunctional agents with good metal chelation, antioxidant, and anti-fibrillation activities. As part of this work, a new assay method was developed to examine the complexation of Zn(II) and Cu(II). The bioavailability and brain uptake of the food constituents was considered via a literature review, and this helped to identify compounds worthy of further investigation in relation to AD.

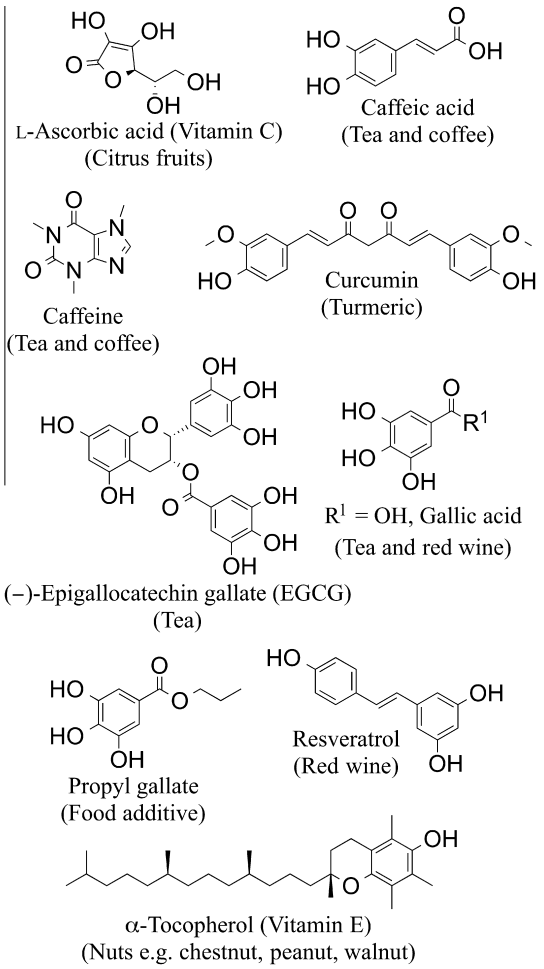


**Fig. 1.** Chemical structures of the food constituents examined in this study, and their main dietary sources. The synthetic non-food-related molecule gallamide ($R^1 = NH_2$) was included in the study to assist in understanding structure–activity relationships.

## 2. Materials and methods

### 2.1. Materials and general methods

The synthetic non-food related molecule gallamide was included in these studies to help to decipher structure–activity relationships, and the known metal chelators EDTA and D-penicillamine were used as positive control comparator compounds in the metal chelation assays. All experiments were conducted at room temperature (23 °C) unless otherwise specified. Commercial chemicals and reagents of at least analytical grade were used. Caffeic acid, curcumin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-(5-bromo-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl) amino]phenol disodium salt dihydrate (5-Br-PAPS), 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine monosodium salt hydrate (ferrozine), iron (II) sulfate heptahydrate, zinc sulfate heptahydrate, EGCG, gallic acid, propyl gallate, 3,4,5-trihydroxybenzamide (gallamide), α-tocopherol, resveratrol, D-penicillamine, caffeine, EDTA, thioflavin T (ThT) and sodium chloride were purchased from Sigma–Aldrich. L-Ascorbic acid was obtained from Chem-Supply. Methanol (HPLC grade) was purchased from Burdick and Jackson. Copper (II) sulfate pentahydrate was obtained from Scharlab. Synthetic $A\beta_{42}$ with purity >95% was purchased from Mimotopes and stored at −80 °C. Type 1 ultrapure water was used throughout the study. Buffers were prepared by reference to ChemBuddy Buffer Maker software (version 1.0.1.55), using disodium hydrogen phosphate and sodium dihydrogen phosphate dihydrate, or HEPES and HEPES sodium salt, purchased from Sigma–Aldrich, and buffers were filtered through a 0.45 μm regenerated cellulose membrane before use. The buffer ionic strength (*I*) was adjusted with NaCl for use at 37 °C. Bath sonication was conducted using a 70 W Soniclean 160HD benchtop ultrasonic cleaner operating at 43 kHz ± 2 kHz sweep bandwidth with 20 Hz pulses. Ultracentrifugation was carried out using a Beckman Coulter Optima L-100 K Preparative Ultracentrifuge with a Type 100 Ti fixed angle rotor and 2 mL quickseal, bell-top polypropylene tubes. UV absorbance was measured using a Cary 50 UV visible spectrophotometer and a 1 cm path length quartz cell, or a Bio-Rad iMark Microplate Absorbance Reader. Fluorescence intensity was measured using a BMG Fluostar Omega plate reader. For the ThT assay, protein LoBind Eppendorf tubes and Corning non-binding surface assay plates (96 well half area black with clear flat bottom polystyrene NBS™ microplate) were used to minimise $A\beta_{42}$ binding to surfaces. To minimise evaporation, the plates were sealed with Corning non-sterile aluminium sealing tape. Copper grids, 200 mesh square for transmission electron microscopy (TEM), were purchased from ProSciTech. Corning polystyrene assay plates (96 well clear flat bottom polystyrene microplates) were used for metal chelation and antioxidant kinetics studies. Regression and statistical analysis was performed using GraphPad Prism 6 (version 6.04). The CLogP of α-tocopherol was calculated using the CLogP function of ChemBioDraw Ultra 13.0; CLogP licensed from BioByte.