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***Sideritis scardica* extracts inhibit aggregation and toxicity of amyloid- β in *Caenorhabditis elegans* used as a model for Alzheimer's disease**

Felix Heiner ^{Corresp., 1}, **Björn Feistel** ², **Michael Wink** ¹

¹ Institute of Pharmacy and Molecular Biotechnology, Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany

² Finzelberg GmbH & Co. KG, Andernach, Germany

Corresponding Author: Felix Heiner
Email address: felixheiner@gmail.com

Background. Beyond its traditional uses in the Balkan area, *Sideritis scardica* (known as Greek mountain tea, Lamiaceae) is currently extensively investigated for its pharmacological activity in the central nervous system. Antidepressant, psychostimulating, cognition-enhancing and neuroprotective properties have been described. In this study, we tested hydroalcoholic extracts of *S. scardica* for their potential to counteract amyloid- β toxicity and aggregation, which plays a crucial role in the pathogenesis of Alzheimer's disease.

Methods. For this purpose, we have chosen the nematode *Caenorhabditis elegans*, which is used as a model organism for neurodegenerative diseases. The concentration of different polyphenols in extracts prepared from water, 20, 40, 50, and 70 % ethanol was analysed by HPLC. Additionally, polar and unpolar fractions were prepared from the 40 % ethanolic extract and phytochemically analysed.

Results. Essentially, the contents of all measured constituents increased with the lipophilicity of the extraction solvents. Treatment of transgenic *C. elegans* strains expressing amyloid- β with the extracts resulted in a reduced number of peptide aggregates in the head region of the worms and alleviated toxicity of amyloid- β , observable through the degree of paralysed animals. The mid-polar extracts (40 and 50 % ethanol) turned out to be the most active, decreasing the plaque number by 21 % and delaying the amyloid- β -induced paralysis by up to 3.5 h. The more lipophilic extract fractions exhibited higher activity than the hydrophilic ones.

Discussion. *Sideritis scardica* extracts demonstrated pharmacological activity against characteristics of Alzheimer's disease also in *C. elegans*, supporting current efforts to assess its potential for the treatment of cognitive decline. The active principle as well as the mode of action needs to be investigated in more detail.

1 ***Sideritis scardica* extracts inhibit aggregation and toxicity of amyloid- β in**
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3 Felix Heiner ¹, Björn Feistel ², Michael Wink ¹

4 ¹ Institute of Pharmacy and Molecular Biotechnology, Department of Biology,
5 Heidelberg University, INF 364, 69120 Heidelberg, Germany

6 ² Finzelberg GmbH & Co. KG, Koblenzer Str. 48-56, 56626 Andernach, Germany

7 Corresponding author:

8 Felix Heiner ¹

9 E-mail address: felixheiner@gmail.com

10 Abstract

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27 by 21 % and delaying the amyloid- β -induced paralysis by up to 3.5 h. The more lipophilic extract
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31 potential for the treatment of cognitive decline. The active principle as well as the mode of action
32 needs to be investigated in more detail.

33 Abbreviations

34	A β :	amyloid- β
35	ACN:	acetonitrile
36	AD:	Alzheimer's disease
37	APP:	amyloid precursor protein
38	DAD:	diode array detector
39	DER:	drug-extract ratio
40	EGCG:	(-)-epigallocatechin-3-gallate
41	EtOH40:	40 % ethanolic <i>S. scardica</i> extract
42	HMPC:	Committee on Herbal Medicinal Products
43	MCI:	mild cognitive impairment
44	Rf:	retardation factor

45 Introduction

46 Alzheimer's disease (AD) is the most common type of dementia and also the most common
 47 neurodegenerative disorder in general. As nowadays more people reach a high age than in the
 48 past and a cure is still missing, AD is a rising concern for modern civilizations. According to the
 49 World Alzheimer Report 2015, about 47 million people suffered from dementia in 2015, and
 50 numbers may double every 20 years (Prince et al., 2015). A variety of possible causes for AD are
 51 being discussed, of which amyloid- β peptides ($A\beta$) still play a key role in Alzheimer research and
 52 which could be targeted by drugs therapeutically or preventively (Hardy & Selkoe, 2002). $A\beta$ is
 53 derived from the amyloid precursor protein (APP) by cleavage through β - and γ -secretases
 54 (Selkoe, 1997). The monomers aggregate to oligomers, to polymers and finally to senile plaques,
 55 which are abundant in the brain of patients suffering from AD (Lansbury, 1999). The traditional
 56 formulation of the amyloid hypothesis blamed those mature aggregates for neurodegeneration,
 57 but the smaller oligomers were discovered to be the most neurotoxic $A\beta$ species (Lambert et al.,
 58 1998; Walsh & Selkoe, 2004).

59 *Sideritis scardica* Griseb. (Lamiaceae) is a perennial shrub endemic to the Balkan peninsula, with
 60 Bulgaria as its main habitat. Depending on the area, it is commonly known as Greek mountain
 61 tea, Shepherd's tea, Ironwort, Mursalski tea, Pirinski tea, or Caj Mali. A broad range of traditional
 62 uses of *S. scardica* are known, including the treatment of bronchitis, asthma, sore throat, the
 63 prevention of anemia, and the use as tonic or poultice (Todorova & Trendafilova, 2014).
 64 Concerning the traditional use against cough associated with common cold and gastrointestinal
 65 discomfort, a HMPC (2016) monograph is available. The plant is rich in polyphenols, such as
 66 flavonoids, hydroxycinnamic acid derivatives, and phenylethanoid glycosides (Evstatieva, 2002;
 67 Petreska et al., 2011). Pharmacological activities like antimicrobial, gastroprotective and anti-
 68 inflammatory activity are mostly accredited to this class of secondary metabolites (Tadic et al.,
 69 2007; Tadic et al., 2012a; Tadic et al., 2012b). Recently the effects of *S. scardica* extracts on the
 70 central nervous system were addressed in a number of studies. Hydroalcoholic extracts were able
 71 to inhibit the reuptake of the monoamine neurotransmitters noradrenaline, dopamine and
 72 serotonin *in vitro* (Feistel & Appel, 2013; Knörle, 2012). Furthermore, they showed
 73 antidepressant and psychostimulating effects, as well as a modulation of AMPA-dependent
 74 neurotransmission in rats (Dimpfel, 2013; Dimpfel et al., 2016a). In mice, cognitive enhancement
 75 and $A\beta$ -counteracting effects were observed (Hofrichter et al., 2016). Also, clinical studies have
 76 already been performed. *S. scardica* extracts were able to improve the mental performance of
 77 healthy subjects under stress conditions and of subjects suffering from mild cognitive impairment
 78 (MCI), which is a precursor of AD (Behrendt et al., 2016; Dimpfel et al., 2016b). A double-blind,
 79 randomized, and placebo-controlled clinical trial currently demonstrated a significant effect of a
 80 combination of *Sideritis scardica* and *Bacopa monnieri* extract (memoLoges®) on the mental
 81 performance of subjects suffering from MCI (Dimpfel et al., 2016c).

82 To further investigate the influence of hydroalcoholic *S. scardica* extracts on neurodegenerative
 83 diseases and especially on $A\beta$ toxicity and aggregation, we have chosen *Caenorhabditis elegans*
 84 as a model organism (Link, 2006). In transgenic strains expressing human $A\beta$ (1-42), *in vivo*
 85 effects can be observed, that, unlike *in vitro* studies, also consider bioavailability and other
 86 biological influences on a multicellular organism. In the present study, we also tried to figure out

the influence of extraction solvents on the content of polyphenolic compounds and pharmacological activity, if a dose-dependency exists, and which extract fractions are potent in order to explore the active principle.

Materials and Methods

Plant material

The drug *Sideritidis scardicae herba* from cultivation in Bulgaria was obtained from Finzelberg GmbH & Co. KG, Andernach, Germany (Item 2232000; Batch 10018839). Voucher specimens are deposited at the Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany (registration number P8562) and at the Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Greece (Specimen-No. PAS101), where the plant material was identified and specified. Five crude extracts with water, ethanol 20 %, 40 %, 50 % and 70 % (V/V) were prepared by exhaustive extraction with twofold moved maceration. After filtering and uperisation (3 sec at 120 °C) they were dried under vacuum. The 40 % ethanolic extract was additionally fractionated through liquid-liquid extraction (aqueous and butanolic phase), reprecipitation in 70 % ethanol (V/V) (supernatant and precipitate) and solid-liquid separation with an Amberlite® XAD7HP (Sigma-Aldrich, St. Louis, USA) adsorber resin (aqueous and ethanolic phase). All test substances were stored at 4 °C.

Phytochemical analysis

The extracts and fractions were analyzed for total polyphenols with a Folin-Ciocalteu UV method following chapter 2.8.14. of the European Pharmacopoeia (2017) and for specific polyphenolic compounds (flavonoids, acteoside, caffeoylquinic acids) with a HPLC method. For this purpose, a Luna® C18/2 column (Phenomenex, Torrance, CA, USA; 250 mm length, 4.6 mm inner diameter, 5 µm particle size) was used at a temperature of 40 °C in a Shimadzu LC10 HPLC system. 10 µL of about 5 mg/mL sample were injected. The mobile phase was composed of water + 0.1 % H₃PO₄ (H₂O) and acetonitrile + 0.1 % H₃PO₄ (ACN) with the following gradient: From 95 % H₂O / 5 % ACN (0 min) to 50 % H₂O / 50 % ACN in 41 min; 100 % ACN from 45 to 50 min to 95 % H₂O / 5 % ACN until 52 min; 65 min in total. The compounds were detected by DAD at 330 nm and calculated through scutellarin and acteoside (Phytolab, Vestenbergsgreuth, Germany) as external standards. Additionally thin layer chromatography (TLC) was conducted to highlight differences of the fractions. As the stationary phase, silica gel 60 F₂₅₄ was used. The plate was cleaned and activated with ethyl acetate/ methanol 50:50 (V/V) and dried at 105 °C for 30 min. 10 µL of preparations from 1 g *S. scardica* extract and 10 mL ethanol 50 % (10 min at 65 °C, filtered) were applied and separated within 15 cm using dichloroethane/ acetic acid/ methanol/ water 50:25:15:10 (V/V/V/V) as mobile phase. After drying, anisaldehyde solution R (European Pharmacopoeia, 2017) was sprayed on the plate, which was dried again for 3 min at 120 °C.

124 *C. elegans* culture conditions

125 All strains were obtained from the Caenorhabditis Genetics Center. The worms were cultured on
126 nematode growth medium (NGM) with *E. coli* OP50 as a food source at 20 °C (strains CL2006,
127 NL5901) or 16 °C (CL4176, CL802). To start with age-synchronized worms, a hypochlorite
128 treatment of gravid adults, which isolates the eggs, was performed before every assay (1 %
129 NaOCl, 0.5 M NaOH; Sigma-Aldrich, St. Louis, MI, USA).

130 Quantification of β -amyloid aggregates

131 Isolated eggs of strain CL2006 were incubated in S-medium containing about 10^9 *E. coli*
132 OP50/mL for 48 h at 16 °C. The hatched worms were then transferred to NGM plates containing
133 the desired concentration of the test substances and *E. coli* OP50. 100 μ g/mL EGCG (Sigma-
134 Aldrich, St. Louis, MI, USA) from green tea served as a positive control. After 96 h of incubation
135 at 16 °C the worms were fixed and A β aggregates were stained with 0.0125 % thioflavin S
136 (Sigma-Aldrich, St. Louis, MI, USA) in 50 % ethanol as described before (Fay et al., 1998). The
137 A β plaques in the head region of 20-25 worms per treatment were counted using a Keyence BZ-
138 9000 fluorescence microscope with a GFP filter (excitation wavelength 480 nm, emission
139 wavelength 510 nm).

140 Paralysis assay (A β toxicity)

141 The assay was performed as described before (Dostal & Link, 2010). In brief, the treated worms
142 were kept at 16 °C for 48 h, then the temperature was upshifted to 25 °C to induce the expression.
143 On the next day, scoring was conducted at least every 2 h for at least 12 h. The worms were
144 counted as paralysed if they failed to respond to several touches with a platinum wire.

145 Statistical analysis

146 All results are expressed as the mean \pm S.E.M of at least three independently repeated
147 experiments. The median paralysis times (PT₅₀) were obtained with a Kaplan-Meier survival
148 analysis. One-way ANOVA with Bonferroni *post-hoc* correction/ independent two-sample
149 Student's *t*-tests (equal variance) were carried out to analyze statistical differences (as
150 appropriate).

151 Results

152 The phytochemical analysis revealed that basically the content of all tested plant compounds
153 increased with decreasing polarity of the extraction solvent (**Table 1**). Nevertheless, compared to
154 extraction solvents of stronger lipophilicity, the amount of total phenols in the 40 % ethanolic
155 extract was surprisingly high. From water to 70 % ethanol the content of acteoside was enriched

almost 13-fold in concentration. The more lipophilic fractions of the 40 % ethanolic primary extract (Liq-Liq BuOH, Reprecip. supernat., Resin EtOH) showed higher concentrations of total polyphenols, acteoside and caffeoylquinic acids than the polar ones (Liq-Liq H₂O, Reprecip. precip., Resin H₂O) and also compared to the primary extract (**Figure 1**). The butanolic fraction of the liquid-liquid extraction contained especially high yields of the analyzed polyphenolic compounds. It contained concentrations of acteoside and flavonoids that were approximately three times higher than the primary extract. Taking the distribution of mass into consideration, acteoside especially seemed to selectively accumulate in lipophilic solvents used for fractionation. The amount of flavanoids in the fractions obtained from reprecipitation in 70 % ethanol constituted the only case of a lower concentration in the unpolar fraction (supernatant) compared to the polar one (precipitate) and to the primary extract. However, the TLC consistently displays the generally higher content of polyphenolic constituents in the lipophilic fractions (flavonoids R_f 0,3 – 0,5 and tannins R_f 0,7 -0,8) in comparison to those with stronger polarity and to the original extract (**Figure 2**).

The transgenic *C. elegans* strain CL2006 constitutively expresses human A β (1-42) (Link, 1995). These peptides form aggregates, which were stained with thioflavin S for quantification; **Figure 3** shows the visualized plaques in the head region of the worms (**A**). In a concentration of 600 μ g/mL all *Sideritis scardica* extracts significantly reduced the number of A β aggregates (**B**). The extract made of 20 % ethanol clearly showed a concentration-dependent activity (**C**), whereas the treatment with 50 % ethanolic extract seemed to lose effectiveness when the concentration was raised from 400 to 600 μ g/mL. Worms that were treated with 400 μ g/mL of lipophilic fractions prepared from the 40 % ethanolic extract showed similar plaque numbers to the actual primary extract in the same concentration (**D**). In contrast, the hydrophilic fractions showed weak or no significant activity (water phase of resin separation: 11 % reduction). Taken together, the lowest numbers of A β plaques were seen in worms treated with 400 and 600 μ g/mL 40 % and 400 μ g/mL 50 % ethanolic extract (about 21 % reduction), which was slightly better than the positive control EGCG (19 %) studied previously in our laboratory (Abbas & Wink, 2010).

The temperature-inducible expression of human A β (1-42) makes worms of strain CL4176 paralyse over time, which is an outcome of A β toxicity (Dostal & Link, 2010). The control strain CL802 does not express A β . The progression of this paralysis was traced for at least 12 h (**Figure 4**). The PT₅₀, a median value describing the point in time when exactly 50 % of the worms were paralysed, was calculated to test for statistically significant differences. Extracts and fractions were tested in two sets of experiments, which showed slightly, but not significantly differing values of the control (**Table 2**). Nevertheless, all treatments were compared to the respective control of the test series. All worms treated with 600 μ g/mL of the different *S. scardica* extracts showed a delay of the A β -induced paralysis similar to or better than the positive control 100 μ g/mL EGCG. The most active extract was the one prepared from 50 % ethanol (more than 10 % delay), which was also acting in a concentration-dependent manner (**Figure 4, B**). 600 μ g/mL of the 40 % ethanolic extract showed about 5 % delay, but was tested in another series of experiments, which makes a direct comparison inappropriate, especially as the percentage delay of EGCG also differs from 6 to 3 % in the two sets. Treatment with 400 μ g/mL of the more lipophilic extract fractions attenuated the progression of the A β -induced paralysis (about 6 %

198 delay each), whereas the polar fractions failed to increase the PT₅₀ significantly (**Table 2; Figure**
199 **4, C**). Worms of the control strain (not expressing A β) that were also treated with the extracts or
200 fractions in the highest used concentration, did not exhibit any paralysis.

201 Discussion

202 All *S. scardica* extracts tested significantly reduced the number of A β aggregates and alleviated
203 A β toxicity in transgenic *C. elegans* strains in a concentration of 600 μ g/mL or less. Taken
204 together, the 40 and 50 % ethanolic extracts were the most active, although EtOH40 did not show
205 a similar percentage delay of paralysis. But a direct comparison of the values is difficult, as they
206 were obtained from two different test series that were performed about 1.5 years apart. In an *in*
207 *vivo* system like *C. elegans* a lot of factors, including behavior, can change to some extent. The
208 worms of the second set basically started to paralyse some hours later and showed slightly
209 different paralysis progression (see **Figure 4**). But obviously data are consistent within the sets
210 and none of the active substances tested in the same set showed a significantly higher activity
211 than EtOH40. Thus, compared to extracts prepared from solvents of higher or lower polarity, the
212 two mid-polar extracts showed the strongest activity, although they did not contain the highest
213 content of flavonoids, acteoside, and caffeoylquinic acids as polyphenolic lead compounds.
214 Reasons for this may be based on pharmacodynamic synergisms of certain extract constituents, as
215 plant extracts always embody multicomponent mixtures. To elucidate this question, further
216 studies must be performed. Also, bioavailability of active compounds or even bioenhancing
217 effects may play a role. Polyphenolics contained in *S. scardica* were reported to be bioavailable,
218 as in a clinical study 5 % of polyphenols ingested with a cup of tea were found as metabolites in
219 urine samples via a HPLC-MS measurement (Petreska & Stefova, 2013). Drug absorption is not
220 very well investigated in *C. elegans* itself, but doubtless compounds have to show similar
221 properties as in vertebrates to be well absorbed from the intestine. According to Zheng et al.
222 (2013) the amount of drugs being absorbed is similar in *C. elegans* and mice. Furthermore, the
223 specificity of extraction solvents regarding the ratio of active to inactive constituents could lead to
224 higher activity of the mid-polar extracts.

225 The 20 and 50 % ethanolic extracts were tested in different concentrations, showing a dose-
226 dependence in both assays. Only worms of strain CL2006 treated with 600 μ g/mL EtOH50 did
227 not show a lower number of A β plaques compared to 400 μ g/mL. Beginning toxic effects at this
228 concentration are highly improbable, as all chosen treatments were tested for their toxicity on *C.*
229 *elegans* (data not shown). More likely, the extract is exhibiting a U-shaped dose-response curve,
230 that is more realistic in biological systems than linear responses (Calabrese & Baldwin, 2001).

231 All the more lipophilic fractions of the primary 40 % ethanolic extract showed a significant
232 reduction in number of A β aggregates as well as a delayed A β -induced paralysis, with the level of
233 activity being similar to the primary extract or just slightly higher, which points out that the
234 lipophilicity of the extract constituents is perhaps not important alone, otherwise the extract
235 prepared from 70 % ethanol would have also shown better results than the mid-polar extracts.
236 However, in most cases the polar fractions did not reveal significant effects, but show trends. So,
237 synergistic effects, maybe of polar and unpolar constituents, are still worth being discussed and

investigated. Considering the phytochemical profile of the extract fractions, it is not completely clear if their A β -counteracting activities can be attributed to their content of total polyphenols, or to a more specific class of compounds. But as the precipitated fraction of the reprecipitation of EtOH40 in 70 % ethanol that contained more flavonoids than its lipophilic counterpart always showed lower activity, this group of compounds may not play a central role. Contemplating the enrichment of acteoside in the unpolar fractions, this phenylethanoid glycoside remains the most promising compound for a potential causal correlation of content and activity.

The A β -counteracting activity of hydroalcoholic *S. scardica* extracts has already been shown in mice (Hofrichter et al., 2016). Here the number of A β depositions, as well as the level of soluble A β (1-42) was decreased, which is coherent with the results of the present study. Hofrichter et al. also provided some evidence about the mode of action. They found an intensified A β clearance via enhancement of phagocytosis in microglia and induction of ADAM10 expression, a crucial α -secretase, which cleaves A β (Esch et al., 1990). An induction of ABC transporter could not be found (Hofrichter et al., 2016). An influence of *S. scardica* on secretases cannot be discussed using the results of the present study as the worms were expressing A β through a minigene, not by processing APP. Other possible mechanisms of action against A β toxicity involve anti-inflammatory and antioxidant activities (Gilgun-Sherki et al., 2001; Heneka et al., 2015; Kadowaki et al., 2005; Shelat et al., 2008). As *S. scardica* has already shown anti-inflammatory properties (Tadic et al., 2007), the inhibition of neuroinflammation is a possible mechanism in vertebrates. But as the nematodes are lacking important structures and mediators which promote inflammation, this is not applicable in the chosen model. Also, several antioxidant activities of Greek mountain tea have been described *in vitro* (Todorova & Trendafilova, 2014), but no antioxidant effects of the extracts or fractions, including the level of intracellular ROS (reactive oxygen species) and defense against the pro-oxidant compound juglone, was observed in *C. elegans* (data not shown). *S. scardica* is rich in polyphenols, which makes a direct interaction with A β peptides highly probable, as this is described for many polyphenolic compounds (Porat et al., 2006; Stefani & Rigacci, 2013). Assembly of peptides can be inhibited by hydrogen or ionic bonds (hydroxyl groups of polyphenols and amino groups of peptides), or by hydrophobic interactions. This direct inhibition of A β aggregation and oligomerisation is also described for EGCG, which was used as the positive control (Abbas & Wink, 2010; del Amo et al., 2012; Wang et al., 2010). A reduced oligomerisation could likewise explain the alleviated A β toxicity.

Conclusions

In conclusion, it can be stated that hydroethanolic *Sideritis scardica* extracts inhibit A β aggregation and toxicity in *C. elegans* with the mid-polar extracts being the most active. This augments existing evidence and makes *S. scardica* highly interesting for the treatment or prevention of neurodegenerative diseases like Alzheimer's. Acteoside, a phenylethanoid glycoside, represents a promising, potentially active substance in the extracts and fractions. Nonetheless, further steps have to be taken to investigate the active principle of the extracts and potential synergistic actions of its constituents. In addition, a detailed mechanism of action cannot

277 be stated at the moment; the hypothesized direct inhibition of A β aggregation needs further
278 elucidation.

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Table 1(on next page)

Phytochemical analysis of the *S. scardica* extracts prepared with different solvents.

Abbreviation	H ₂ O	EtOH20	EtOH40	EtOH50	EtOH70
Extraction solvent	Water	20 % ethanol (V/V)	40 % ethanol (V/V)	50 % ethanol (V/V)	70 % ethanol (V/V)
DER native	5.8 : 1	7.2 : 1	4.7 : 1	5.7 : 1	5.7 : 1
Polyphenols [%]	5.07	6.25	9.28	6.23	7.37
Flavonoids [%]	0.59	1.18	2.42	2.03	2.82
Acteoside [%]	0.12	0.41	1.41	0.94	1.54
Caffeoylquinic acids [%]	0.24	0.47	0.39	0.38	0.49

Table 2 (on next page)

Delay of A β -induced paralysis in *C. elegans* strain CL4176.

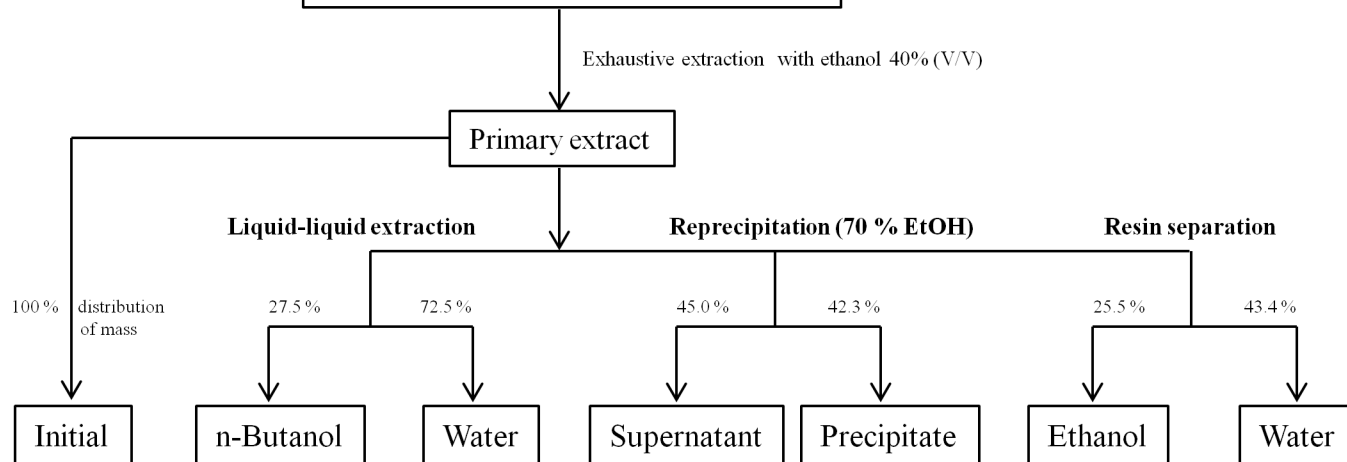
PT₅₀ are median values describing the point in time, when exactly 50 % of the worms are paralysed. Experiments were conducted in different test series with slightly differing values of the control; p values compared to the respective control.

Series of experiments	Treatment	Concentration $\mu\text{g/mL}$	$\text{PT}_{50} \pm \text{S.E.M. [h]}$	Significance
Set 1	Control (0.5 % ethanol)		33.5 ± 0.5	
	EGCG	100	35.5 ± 0.5	$p < 0.05$
	H ₂ O	600	35.5 ± 0.5	$p < 0.05$
	EtOH20	600	35.0 ± 0.6	$p < 0.05$
		800	35.5 ± 0.5	$p < 0.05$
		1000	35.8 ± 0.6	$p < 0.05$
	EtOH50	200	35.0 ± 0.6	
		400	35.5 ± 0.5	$p < 0.05$
		600	37.0 ± 0.0	$p < 0.001$
	EtOH70	600	36.3 ± 0.3	$p < 0.01$
Set 2	Control (0.5 % ethanol)		34.8 ± 0.3	
	EGCG	100	35.8 ± 0.3	$p < 0.05$
	EtOH40	400	35.5 ± 0.3	
		600	36.5 ± 0.3	$p < 0.01$
	Liq-Liq BuOH	400	37.0 ± 0.6	$p < 0.05$
	Liq-Liq H ₂ O	400	35.3 ± 0.3	
	Reprecip. supernat.	400	36.8 ± 0.3	$p < 0.01$
	Reprecip. precip.	400	35.5 ± 0.3	
	Resin EtOH	400	37.0 ± 0.4	$p < 0.01$
	Resin H ₂ O	400	35.3 ± 0.3	

Figure 1(on next page)

Flowchart and phytochemical analysis of the fractions prepared from 40 % ethanolic *S. scardica* extract.

Percentage distribution of mass is given for each pair of fractionation type in the flowchart above. Occurring differences to 100% are loss of preparative handling.



Abbreviation	EtOH40	Liq-Liq BuOH	Liq-Liq H ₂ O	Reprecip. supernat.	Reprecip. precip.	Resin EtOH	Resin H ₂ O
DER native	4.7 : 1	17.1 : 1	6.5 : 1	10.4 : 1	11.1 : 1	18.8 : 1	10.8 : 1
Polyphenols [%]	9.28	15.60	5.42	11.80	6.74	12.30	5.00
Flavonoids [%]	2.42	6.96	0.56	0.97	4.10	3.25	0.67
Acteoside [%]	1.41	4.30	0.19	2.54	0.39	2.36	0.41
Caffeoylquinic acids [%]	0.39	0.50	0.37	0.58	0.26	0.77	0.25

Figure 2 (on next page)

Thin layer chromatogram of polar constituents of 40 % ethanolic *S. scardica* extract and its fractions after spraying with anisaldehyde solution R.

Allocation of the tracks: primary extract with 40 % ethanol (1); butanolic (2) and aqueous (3) phase of liquid-liquid extraction; supernatant (4) and precipitated fraction (5) of reprecipitation in 70 % ethanol; ethanolic (6) and aqueous (7) phase of resin separation.

EtOH40 BuOH H₂O supernat. precip. EtOH H₂O

Rf

Rf

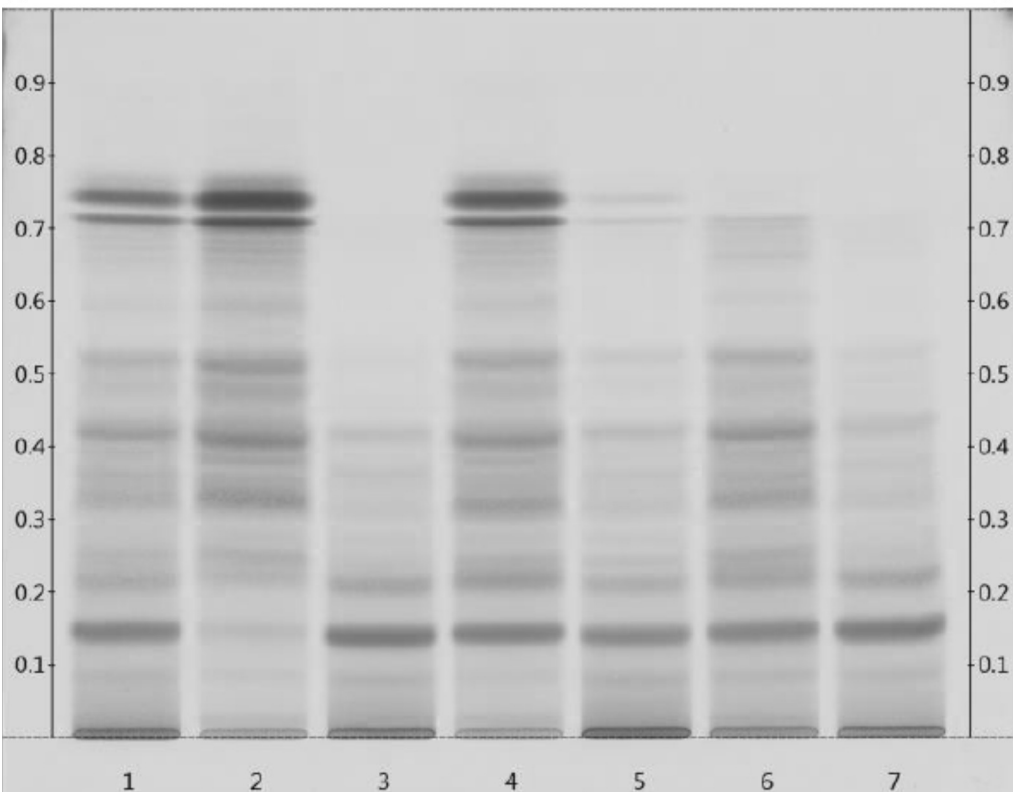


Figure 3(on next page)

Effect of *Sideritis scardica* on A β aggregation in *C. elegans*.

A Fluorescence microscopic image of the head region of a worm from strain CL2006.

Arrowheads point out the β -amyloid plaques that were stained with thioflavin S. **B, C, D**

Reduction in number of the A β aggregates. All extracts were tested in a concentration of 600 μ g/mL compared to 100 μ g/mL EGCG as a positive control (**B**); the 20 % and 50 % ethanolic extract were tested in additional concentrations to show dose-dependence (**C**). All fractions were tested in a concentration of 400 μ g/mL compared to the original 40 % ethanolic extract in the same concentration (**D**). Controls were treated with 0.5 % ethanol. * $p < 0.05$; *** $p < 0.001$; concentrations in μ g/mL

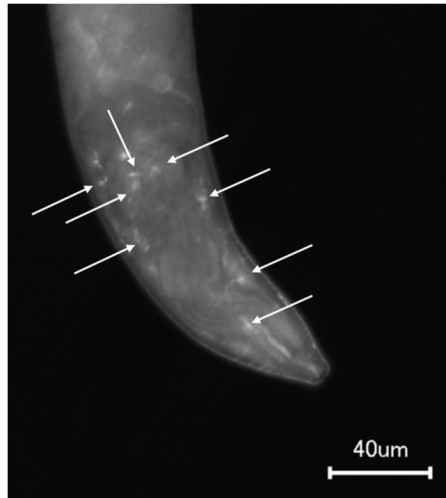
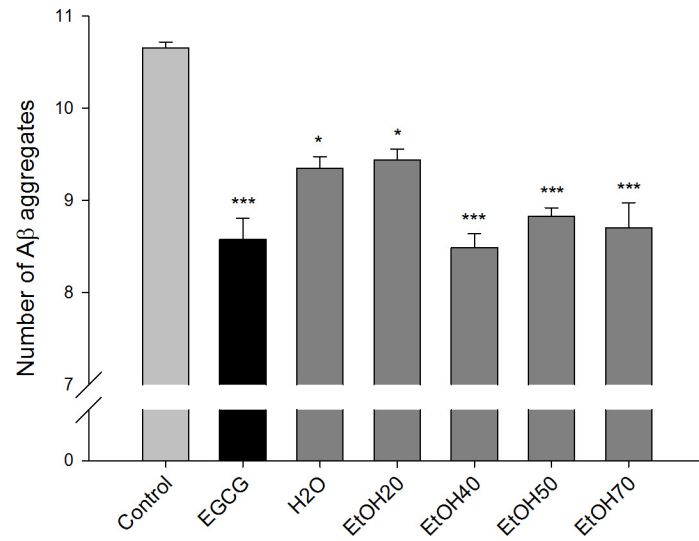
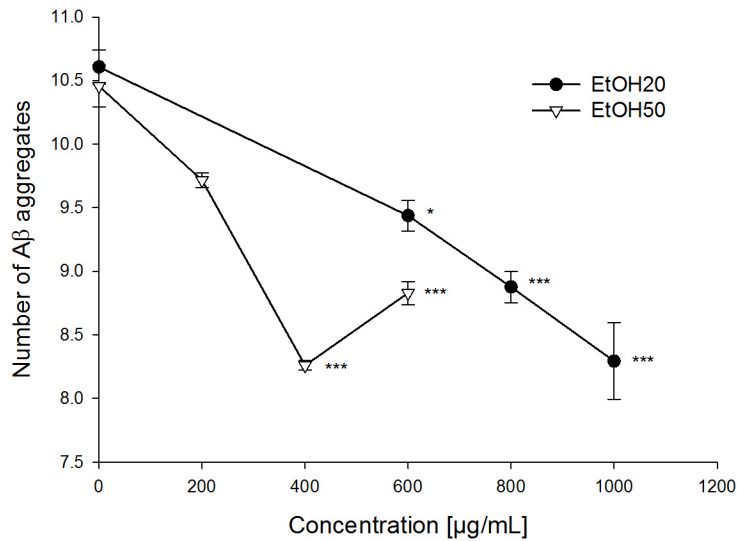
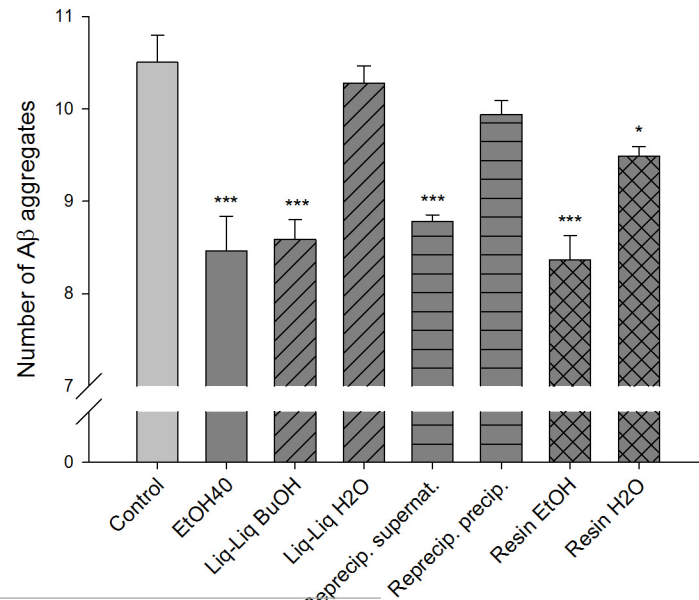
A**B****C****D**

Figure 4(on next page)

Influence of *S. scardica* on A β -induced paralysis.

A, B Paralysis curves from the first set of experiments. Compared to the control (0.5 % ethanol) CL4176 worms treated with 600 μ g/mL extract prepared from water, 20, 50, and 70 % ethanol were paralyzing slower and to a lesser extent (**A**). The control strain, which is not expressing A β , did not show any paralysis. Amongst others, the 50 % ethanolic extract showed a concentration-dependent activity (**B**). **C** Paralysis curves from the second set of experiments. All worms treated with 400 μ g/mL of the more lipophilic extracts showed a delayed A β -induced paralysis, whereas 400 μ g/mL of the polar ones failed to clearly shift the curve to the right. Results of the fractions from liquid-liquid extraction as an example.

