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Supplementary Materials

Neuroprotective Effect of *Yucca schidigera* Roezl ex Ortgies Bark Phenolic Fractions, Yuccaol B and Gloriosaol A on Scopolamine-Induced Memory Deficits in Zebrafish

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Abstract: *Y. schidigera* contains a number of unusual polyphenols, derivatives of resveratrol and naringenin, called spiro-flavostilbenoids, which have potent in vitro anti-inflammatory, antioxidant, and moderate cholinesterase inhibitory activities. To date, these compounds have not been tested in vivo for the treatment of neurodegenerative diseases. The aim of the present study was to evaluate the effects of both single spiro-flavostilbenoids (yuccaol B and gloriosaol A) and phenolic fractions derived from *Y. schidigera* bark on scopolamine-induced anxiety and memory process deterioration using a *Danio rerio* model. Detailed phytochemical analysis of the studied fractions was carried out using different chromatographic techniques and Nuclear Magnetic Resonance (NMR). The novel tank diving test was used as a method to measure zebrafish anxiety, whereas spatial working memory function was assessed in Y-maze. In addition, acetylcholinesterase/butyrylcholinesterase (AChE/BChE) and 15-lipooxygenase (15-LOX) inhibition tests were performed in vitro. All pure compounds and fractions under study exerted anxiolytic and procognitive action. Moreover, strong anti-oxidant capacity was observed, whereas weak inhibition towards cholinesterases was found. Thus, we may conclude that the observed behavioral effects are complex and result rather from inhibition of oxidative stress processes and influence on cholinergic muscarinic receptors (both 15-LOX and scopolamine assays) than effects on cholinesterases. *Y. schidigera* is a source of substances with desirable properties in the prevention and treatment of neurodegenerative diseases.

Keywords: *Yucca schidigera*; Asparagaceae; spiro-flavostilbenoids; stilbenoids; polyphenols; zebrafish; anxiety; memory; cholinergic function; oxidative stress

List of Supplementary Materials

Figure S1. Induced locomotor pattern and behavior in the Y-maze test of the *Yucca schidigera* substances in 3 doses level in scopolamine (Sco)-treated zebrafish. Representative locomotion tracking patterns of the control – untreated group; Sco (100 μM) treated group; groups treated with Sco (100 μM) and yucca (YS) preparations at 1, 3, 5 μg/L concentration levels: (**a**) YS pur – purified fraction; (**b**) YS poly (=YS pro) – polymeric fraction; (**d**) YuB – Yuccaol B; and (**e**) GloA – Gloriosaol A.

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Figure S1. Induced locomotor pattern and behavior in the Y-maze test of the *Yucca schidigera* substances in 3 doses level in scopolamine (Sco)-treated zebrafish. Representative locomotion tracking patterns of the control – untreated group; Sco (100 μM) treated group; groups treated with Sco (100 μM) and yucca (YS) preparations at 1, 3, 5 μg/L concentration levels: (**a**) YS pur – purified fraction; (**b**) YS poly (=YS pro) – polymeric fraction; (**d**) YuB – Yuccaol B; and (**e**) GloA – Gloriosaol A.

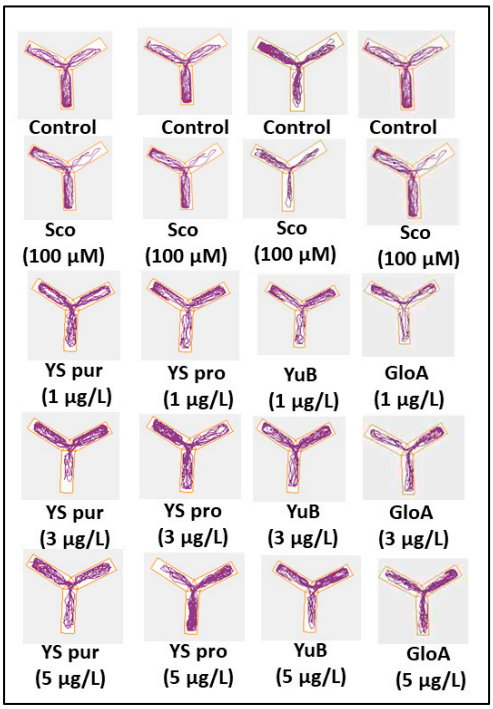


Table S1. Acetylcholinesterase inhibition capacity (%) and EC_{50} (μg/mL) of the *Yucca schidigera* bark phenolic fractions.

Sample	Concentration of sample (mg/mL)								EC_{50}, μg/mL
	0.3125	*0.625*	*1.25*	*2.5*	*5*	*10*	*20*		
YS unpur	5.39 ± 0.07	8.29 ± 0.09	20.55 ± 0.1	38.66 ± 0.08	57.73 ± 0.09	92.14 ± 0.11	96.75 ± 0.08		188.76 ± 0.59
	0.0625	*0.125*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	
YS pur	13.97 ± 0.04	19.80 ± 0.03	21.69 ± 0.07	23.36 ± 0.17	27.99 ± 0.04	32.62 ± 0.17	36.91 ± 0.15	46.17 ± 0.17	419.45 ± 0.86[a]
YS poly	12.09 ± 0.04	17.23 ± 0.05	19.91 ± 0.06	28.37 ± 0.08	34.64 ± 0.09	55.89 ± 0.07	74.64 ± 008	89.09 ± 0.14	82.51 ± 0.20[a]
Gal	16.25 ± 0.08	23.05 ± 0.16	30.58 ± 0.20	50.35 ± 0.15	69.23 ± 0.18	79.95 ± 0.10	92.27 ± 0.12	98.02 ± 0.17	24.69 ± 0.09

Notes: [a]sample vs galantamine $p<0.0001$ (statistically significant). YS unpur – *Y. schidigera* unpurified phenolic fraction; YS pur – *Y. schidigera* purified phenolic fraction; YS poly – *Y. schidigera* polymeric fraction; Gal – galantamine.

Table S2. Butyrylcholinesterase inhibition capacity (%) and EC_{50} (μg/mL) of the *Yucca schidigera* bark phenolic fractions.

Sample	Concentration of sample (mg/mL)								EC_{50}, μg/mL
	0.3125	*0.625*	*1.25*	*2.5*	*5*	*10*	*20*		
YS unpur	12.30 ± 0.15	20.26 ± 0.23	27.30 ± 0.18	41.80 ± 0.21	58.37 ± 0.48	88.81 ± 0.92	93.80 ± 0.25		176.14 ± 1.14
	0.0625	*0.125*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	
YS pur	2.93 ± 0.11	4.88 ± 0.04	7.25 ± 0.11	14.05 ± 0.08	24.69 ± 0.12	34.16 ± 0.09	55.84 ± 0.08	78.77 ± 0.13	165.93 ± 0.44[a]
YS poly	7.28 ± 0.08	15.45 ± 0.10	20.25 ± 0.11	27.46 ± 0.08	36.77 ± 0.10	49.15 ± 0.11	62.63 ± 0.12	90.75 ± 0.18	104.46 ± 0.59[a]
Gal	15.05 ± 0.09	21.85 ± 0.10	31.09 ± 0.11	52.47 ± 0.18	70.69 ± 0.20	81.01 ± 0.17	92.45 ± 0.13	98.29 ± 0.17	23.07 ± 0.13

Notes: [a]sample vs galantamine $p<0.0001$ (statistically extremely significant). YS unpur – *Y. schidigera* unpurified phenolic fraction; YS pur – *Y. schidigera* purified phenolic fraction; YS poly – *Y. schidigera* polymeric fraction; Gal – galantamine.

Table S3. 15-Lipoxygenase inhibition activity (%) and EC_{50} (μg/mL) of the *Yucca schidigera* bark phenolic fractions, yuccaol B, and gloriosaol A.

Sample	Concentration of sample (mg/mL)								EC_{50}, μg/mL
	0.3125	*0.625*	*1.25*	*2.5*	*5*	*10*			
YS unpur	24.34±0.41	33.39±0.33	55.21±0.19	71.16±0.24	92.08±0.51	100±0			17.65±0.13
	0.0625	*0.125*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	
YS pur	9.13 ± 0.33	12.40 ± 0.94	23.08 ± 0.77	48.67 ± 0.61	67.05 ± 1.26	98.93 ± 1.05	100 ± 0	100 ± 0	8.76 ± 0.21[a]
YS poly	9.67 ± 0.46	16.97 ± 0.38	25.55 ± 1.14	35.56 ± 0.80	64.42 ± 0.56	96.40 ± 1.09	100 ± 0	100 ± 0	12.78 ± 1.74[b]
YuB	12.95 ± 0.28	20.2 ± 0.86	29.84 ± 0.94	42.96 ± 0.53	75.67 ± 0.89	98.81 ± 0.68	-*	-*	9.66 ± 0.12[a]
GloA	7.45 ± 0.28	13.14 ± 0.47	25.74 ± 0.88	38.17 ± 0.95	59.04 ± 1.37	76.68 ± 1.65	100 ± 0	100 ± 0	12.34 ± 0.48[a]
Vit. C	10.92 ± 0.80	15.71 ± 0.31	20.84 ± 1.05	30.19 ± 0.79	40.67 ± 1.82	65.89 ± 1.25	94.19 ± 0.98	100 ± 0	21.52 ± 0.95

Notes: *insufficient sample quantity, [a]sample *vs* vitamin C $p<0.0001$ (statistically extremely significant); [b]sample vs vitamin C $p<0.0016$ (statistically highly significant). YS unpur – *Yucca schidigera* unpurified phenolic fraction; YS pur – *Y. schidigera* purified phenolic fraction; YS poly – *Y. schidigera* polymeric fraction.

Table S4. Cholinesterase inhibition (%) and IC_{50} values of *Yucca schidigera* phenolic pure metabolites.

No.	Compound	AChEi [1] (Inhibition % ± S.D. [2]) 1000 μM [3]	BChEi [4] (Inhibition % ± S.D. [2]) 1000 μM [3]
1	THMS [5]	19.33 ± 3.03 %	25.21 ± 2.58 %
2	Dihydrokaempferol	5.09 ± 2.38 %	8.56 ± 2.83 %
3	Naringenin	6.89 ± 1.82 %	21.07 ± 2.44 %
4	Galantamine [6]	97.92 ± 0.01% (IC_{50} = 2.29 ± 0.33 μM)	91.52±1.63 % (IC_{50} = 124.03 ± 4.05 μM)

[1] AChEi – acetylcholinesterase inhibition; [2] S.D. – Standard deviation; [3] Final concentration; [4] BChE – butyrylcholinesterase inhibition; [5] THMS – *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene; [6] reference substance.