

## Supplementary Materials

### **Evaluation of the Impact of *Alternanthera philoxeroides* (Mart.) Griseb. Extract on Memory Deficits in D-Galactose-Induced Brain Aging in Mice Through Effects on Antioxidant Enzymes, Neuroinflammation, and Telomere Shortening**

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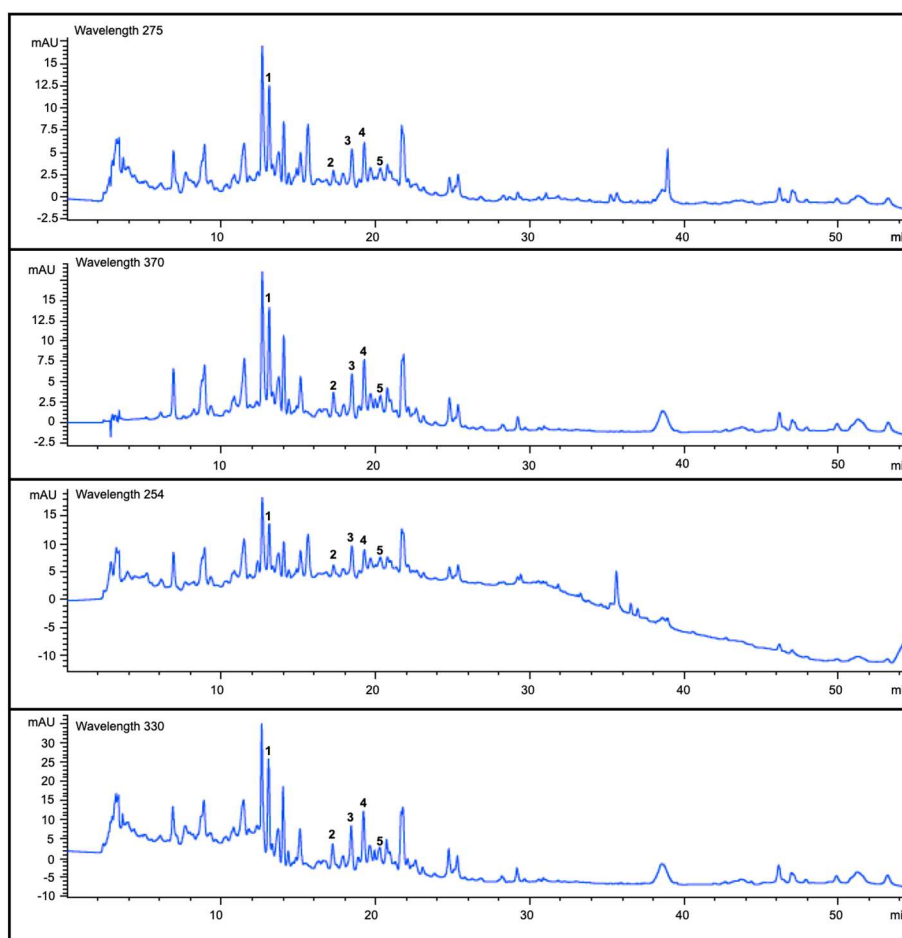
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# 1. Phytochemical Analysis by High-Performance Liquid Chromatography (HPLC) and Validation Method



**Figure S1.** HPLC chromatograms of five standards in the ethanol extract of *A. philoxeroides* (1 = kaempferol-*O*-glucoside, 2 = quercetin, 3 = alternanthin B, 4 = demethyltorosaflavone D, 5 = chrysoeriol-7-*O*-rhamnoside)

**Table S1.** Validation results of the analytical method for determination of phytochemical constituents in the ethanol extract of AP

Parameter	Compounds				
	(1)	(2)	(3)	(4)	(5)
Retention Time (min)	13.10	17.27	18.47	19.27	20.32
Range (µg/mL)	1-10	2.5-25	2.5-25	10-100	2.5-25
Linearity	Coefficient determination (R <sup>2</sup> )				
	0.9993 ± 0.0002	0.9997 ± 0.0000	0.9997 ± 0.0001	0.9998 ± 0.0002	0.9992 ± 0.0007
LOD (µg/mL)	0.2	0.5	0.5	2.0	0.6
LOQ (µg/mL)	0.6	1.0	1.0	7.0	2.9
Precision	Within day				
(%RSD)	0.350 – 1.290	0.950 – 1.430	0.820 – 1.430	1.400 – 2.000	0.531 – 1.13
	Between day				
	0.890 – 1.467	1.210 – 1.810	1.520 – 1.910	1.810 – 1.900	1.630 – 1.680
Accuracy (%Recovery)	108.20 ± 0.812	104.27 ± 0.045	101.538 ± 2.776	102.68 ± 2.765	107.030 ± 2.206
	101.31 ± 3.50	99.09 ± 3.890	96.342 ± 3.207	91.969 ± 5.395	104.490 ± 3.481
	97.97 ± 1.321	99.19 ± 1.270	102.049 ± 1.789	103.55 ± 5.234	99.550 ± 4.340

2. Statistical Analysis Effect of AP extract on D-Gal-Induced Memory Deficits in Behavioral Tests Using Y-maze test

**Table S2.** Statistical test of Y-maze test.

Test for parametric analysis	P	
	The Student's t-test	One-way ANOVA
Normality Test	0.864 (Passed)	0.494 (Passed)
Equal Variance Test	0.116 (Passed)	0.573 (Passed)
Group comparison	The Student's t-test	
Healthy control vs. Vehicle group	P < 0.001	
	ANOVA followed by Tukey's post hoc test	
	P	F (DF <sub>between group</sub> , DF <sub>residual</sub> )
All group	<0.001	F(3,36)=12.367
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.006	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.130	
Vit.E group vs. AP 250 group	0.783	
Vit.E group vs. AP 500 group	0.565	

3. Statistical Analysis Effect of A. philoxeroides extract (AP) on D-Gal-Induced Memory Deficits in Behavioral Tests Using Novel Object Recognition Test (NORT)

**Table S3.** Statistical test of novel object recognition test (NORT).

Test for parametric analysis	P	
	The Student's t-test	One-way ANOVA
Normality Test	0.053 (Passed)	0.682 (Passed)
Equal Variance Test	0.102 (Passed)	0.329 (Passed)
Group comparison	The Student's t-test	
Healthy control vs. D-Gal + Vehicle group	P < 0.001	
	ANOVA followed by Tukey's post hoc test	
	P	F (DF <sub>between group</sub> , DF <sub>residual</sub> )
The test phase trial		
All group	<0.001	F(3,36)=63.094
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	<0.001	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.236	
Vit.E group vs. AP 250 group	0.113	
Vit.E group vs. AP 500 group	0.979	

4. Statistical Analysis Effect of AP extract on D-Gal-Induced Memory Deficits in Behavioral Tests Using Morris Water Maze Test (MWMt)

**Table S4.1** Statistical test of MWMt on the training test

Test for parametric analysis		<i>P</i>	
		The Student's t-test	One-way ANOVA
Day 1-3		-	-
Day 4:	Normality Test	0.641 (Passed)	0.596 (Passed)
	Equal Variance Test	0.549 (Passed)	0.917 (Passed)
Day 5:	Normality Test	0.823 (Passed)	0.715 (Passed)
	Equal Variance Test	0.059 (Passed)	0.687 (Passed)
Group comparison		The Student's t-test	
Day 4: Healthy control vs. D-Gal + Vehicle group		$P < 0.001$	
Day 5: Healthy control vs. D-Gal + Vehicle group		$P < 0.001$	
		ANOVA followed by Tukey's post hoc test	
		<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
Day 1-3		-	-
Day 4: All group		0.007	$F (3,36)=4.740$
Vehicle group vs. Vit.E group		0.925	
Vehicle group vs. AP 250 group		0.593	
Vehicle group vs. AP 500 group		0.088	
AP 250 group vs. AP 500 group		0.040	
Vit.E group vs. AP 250 group		0.254	
Vit.E group vs. AP 500 group		0.282	
Day 5: All group		<0.001	$F (3,36)=12.425$
Vehicle group vs. Vit.E group		0.010	
Vehicle group vs. AP 250 group		0.168	
Vehicle group vs. AP 500 group		<0.001	
AP 250 group vs. AP 500 group		0.003	
Vit.E group vs. AP 250 group		0.600	
Vit.E group vs. AP 500 group		0.062	

**Table S4.2** Statistical test of MWMT on the probe test

Test for parametric analysis	<i>P</i>	
	The Student's t-test	One-way ANOVA
Normality Test	0.636 (Passed)	0.347 (Passed)
Equal Variance Test	0.392 (Passed)	0.061 (Passed)
Group comparison	The Student's t-test	
Healthy control vs. D-Gal + Vehicle group	$P < 0.001$	
	ANOVA followed by Tukey's post hoc test	
	<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
All group	<0.001	$F(3,36)=8.325$
Vehicle group vs. Vit.E group	0.017	
Vehicle group vs. AP 250 group	0.002	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.911	
Vit.E group vs. AP 250 group	0.851	
Vit.E group vs. AP 500 group	0.468	

5. Statistical Analysis AP extract Reversed Activity of Superoxide Dismutase (SOD) and Catalase (CAT) in the Hippocampus and Frontal cortex in the Frontal cortex and Hippocampus

**Table S5.1** Statistical test of AP extract Reversed Activity of Superoxide Dismutase (SOD)

Test for parametric analysis	<i>P</i>	
	The Student's t-test	One-way ANOVA
Hippocampus: Normality Test	0.689 (Passed)	0.158 (Passed)
Equal Variance Test	0.956 (Passed)	0.969 (Passed)
Frontal cortex: Normality Test	0.468 (Passed)	0.178 (Passed)
Equal Variance Test	0.149 (Passed)	0.866 (Passed)
Group comparison	The Student's t-test	
Hippocampus: Healthy control vs. D-Gal + Vehicle group	$P < 0.001$	
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	$P < 0.001$	
	ANOVA followed by Tukey's post hoc test	
	<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
Hippocampus		
All group	0.004	$F(3,20)=11.126$
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.657	
Vehicle group vs. AP 500 group	0.017	
AP 250 group vs. AP 500 group	0.170	
Vit.E group vs. AP 250 group	0.003	
Vit.E group vs. AP 500 group	0.218	

Frontal cortex		
All group	0.012	$F(3,20)=15.773$
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.004	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.291	
Vit.E group vs. AP 250 group	0.246	
Vit.E group vs. AP 500 group	0.999	

**Table S5.2** Statistical test of AP Reversed Activity of Catalase (CAT)

Test for parametric analysis	<i>P</i>	
	The Student’s t-test	One-way ANOVA
Hippocampus: Normality Test	0.165 (Passed)	0.200 (Passed)
Equal Variance Test	0.882 (Passed)	0.877 (Passed)
Frontal cortex: Normality Test	0.119 (Passed)	0.297 (Passed)
Equal Variance Test	0.330 (Passed)	0.657 (Passed)
Group comparison	The Student’s t-test	
Hippocampus: Healthy control vs. D-Gal + Vehicle group	<i>P</i> < 0.001	
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	<i>P</i> < 0.001	
	ANOVA followed by Tukey’s post hoc test	
	<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
Hippocampus		
All group	<0.001	<i>F</i> (3,20)=34.005
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	<0.001	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.016	
Vit.E group vs. AP 250 group	<0.05	
Vit.E group vs. AP 500 group	0.647	
Frontal cortex		
All group	0.004	<i>F</i> (3,20)=24.828
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	<0.001	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.291	
Vit.E group vs. AP 250 group	0.532	
Vit.E group vs. AP 500 group	0.968	

6. Statistical Analysis of AP Extract Alleviated Neuroinflammation as well as Delayed Telomere Shortening in the Hippocampus and the Frontal Cortex

**Table S6.1** Statistical test of IL-1 $\beta$  mRNA expression in hippocampus and frontal cortex

Test for parametric analysis	<i>P</i>
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	The Student's t-test		One-way ANOVA
Hippocampus: Normality Test	0.411 (Passed)		0.535 (Passed)
Equal Variance Test	0.331 (Passed)		0.701 (Passed)
Frontal cortex: Normality Test	0.458 (Passed)		0.325 (Passed)
Equal Variance Test	0.149 (Passed)		0.551 (Passed)
Group comparison	The Student's t-test		
Hippocampus: Healthy control vs. D-Gal + Vehicle group	$P < 0.001$		
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	$P < 0.001$		
	ANOVA followed by Tukey's post hoc test		
	$P$	$F$ (DF <sub>between group</sub> , DF <sub>residual</sub> )	
Hippocampus			
All group	<0.001	$F(3,20)=29.79$	
Vehicle group vs. Vit.E group	<0.001		
Vehicle group vs. AP 250 group	0.001		
Vehicle group vs. AP 500 group	<0.001		
AP 250 group vs. AP 500 group	0.007		
Vit.E group vs. AP 250 group	0.013		
Vit.E group vs. AP 500 group	0.993		
Frontal cortex			
All group	<0.001	$F(3,20)=18.332$	
Vehicle group vs. Vit.E group	<0.001		
Vehicle group vs. AP 250 group	0.965		
Vehicle group vs. AP 500 group	<0.001		
AP 250 group vs. AP 500 group	0.002		
Vit.E group vs. AP 250 group	<0.001		
Vit.E group vs. AP 500 group	0.525		

**Table S6.2** Statistical test of IL-6 mRNA expression in hippocampus and frontal cortex

Test for parametric analysis	$P$	
	The Student's t-test	One-way ANOVA
Hippocampus: Normality Test	0.468 (Passed)	0.768 (Passed)
Equal Variance Test	0.076 (Passed)	0.054 (Passed)
Frontal cortex: Normality Test	0.657 (Passed)	0.261 (Passed)
Equal Variance Test	0.126 (Passed)	0.901 (Passed)
Group comparison	The Student's t-test	
Hippocampus: Healthy control vs. D-Gal + Vehicle group	$P < 0.001$	
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	$P < 0.001$	
	ANOVA followed by Tukey's post hoc test	
	$P$	$F (DF_{\text{between group}}, DF_{\text{residual}})$
Hippocampus		
All group	<0.001	$F(3,20)=25.337$

Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.999	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	<0.001	
Vit.E group vs. AP 250 group	<0.001	
Vit.E group vs. AP 500 group	0.340	
Frontal cortex		
All group	<0.001	$F(3,20)=22.017$
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.014	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.074	
Vit.E group vs. AP 250 group	0.053	
Vit.E group vs. AP 500 group	0.998	

**Table S6.3** Statistical test of TNF- $\alpha$  mRNA expression in hippocampus and frontal cortex

Test for parametric analysis	<i>P</i>	
	The Student’s t-test	One-way ANOVA
Hippocampus: Normality Test	0.141 (Passed)	0.373 (Passed)
Equal Variance Test	0.600 (Passed)	0.116 (Passed)
Frontal cortex: Normality Test	0.449 (Passed)	0.368 (Passed)
Equal Variance Test	0.185 (Passed)	0.812 (Passed)
Group comparison	The Student’s t-test	
Hippocampus: Healthy control vs. D-Gal + Vehicle group	<i>P</i> < 0.001	
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	<i>P</i> = 0.007	
	ANOVA followed by Tukey’s post hoc test	
	<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
Hippocampus		
All group	<0.001	<i>F</i> (3,20)=24.101
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.151	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	<0.001	
Vit.E group vs. AP 250 group	0.001	
Vit.E group vs. AP 500 group	0.973	
Frontal cortex		
All group	<0.001	<i>F</i> (3,20)=8.551
Vehicle group vs. Vit.E group	0.004	
Vehicle group vs. AP 250 group	0.899	
Vehicle group vs. AP 500 group	0.005	
AP 250 group vs. AP 500 group	0.023	
Vit.E group vs. AP 250 group	0.020	
Vit.E group vs. AP 500 group	1.000	



**Table S6.4** Statistical test of mTERT mRNA expression in hippocampus and frontal cortex

Test for parametric analysis	<i>P</i>	
	The Student’s t-test	One-way ANOVA
Hippocampus: Normality Test	0.342 (Passed)	0.764 (Passed)
Equal Variance Test	0.373 (Passed)	0.592 (Passed)
Frontal cortex: Normality Test	0.296 (Passed)	0.192 (Passed)
Equal Variance Test	0.970 (Passed)	0.961 (Passed)
Group comparison	The Student’s t-test	
Hippocampus: Healthy control vs. D-Gal + Vehicle group	<i>P</i> = 0.002	
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	<i>P</i> < 0.001	
	ANOVA followed by Tukey’s post hoc test	
	<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
Hippocampus		
All group	<0.001	<i>F</i> (3,20)=13.731
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.997	
Vehicle group vs. AP 500 group	0.015	
AP 250 group vs. AP 500 group	0.024	
Vit.E group vs. AP 250 group	<0.001	
Vit.E group vs. AP 500 group	0.200	
Frontal cortex		
All group	<0.001	<i>F</i> (3,20)=24.592
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.997	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	<0.001	
Vit.E group vs. AP 250 group	<0.001	
Vit.E group vs. AP 500 group	0.688	

**Table S6.5** Statistical test of mTRF1 mRNA expression in hippocampus and frontal cortex

Test for parametric analysis	<i>P</i>	
	The Student's t-test	One-way ANOVA
Hippocampus: Normality Test	0.691 (Passed)	0.844 (Passed)
Equal Variance Test	0.451 (Passed)	0.395 (Passed)
Frontal cortex: Normality Test	0.515 (Passed)	0.619 (Passed)
Equal Variance Test	0.848 (Passed)	0.379 (Passed)
Group comparison	The Student's t-test	
Hippocampus: Healthy control vs. D-Gal + Vehicle group	$P = 0.001$	
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	$P < 0.001$	

	ANOVA followed by Tukey's post hoc test	
	<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
Hippocampus		
All group	0.002	<i>F</i> (3,20)=7.209
Vehicle group vs. Vit.E group	0.003	
Vehicle group vs. AP 250 group	0.828	
Vehicle group vs. AP 500 group	0.024	
AP 250 group vs. AP 500 group	0.134	
Vit.E group vs. AP 250 group	0.022	
Vit.E group vs. AP 500 group	0.803	
Frontal cortex		
All group	<0.001	<i>F</i> (3,20)=10.558
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.505	
Vehicle group vs. AP 500 group	0.006	
AP 250 group vs. AP 500 group	0.112	
Vit.E group vs. AP 250 group	0.008	
Vit.E group vs. AP 500 group	0.578	

**Table S6.6** Statistical test of mTRF2 mRNA expression in hippocampus and frontal cortex

Test for parametric analysis	<i>P</i>	
	The Student's t-test	One-way ANOVA
Hippocampus: Normality Test	0.499 (Passed)	0.079 (Passed)
Equal Variance Test	0.311 (Passed)	0.882 (Passed)
Frontal cortex: Normality Test	0.208 (Passed)	0.128 (Passed)
Equal Variance Test	0.696 (Passed)	0.059 (Passed)
Group comparison	The Student's t-test	
Hippocampus: Healthy control vs. D-Gal + Vehicle group	<i>P</i> < 0.001	
Frontal cortex: Healthy control vs. D-Gal + Vehicle group	<i>P</i> < 0.001	
	ANOVA followed by Tukey's post hoc test	
	<i>P</i>	<i>F</i> (DF <sub>between group</sub> , DF <sub>residual</sub> )
Hippocampus		
All group	<0.001	<i>F</i> (3,20)=20.121
Vehicle group vs. Vit.E group	<0.001	
Vehicle group vs. AP 250 group	0.188	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	0.008	
Vit.E group vs. AP 250 group	<0.001	
Vit.E group vs. AP 500 group	0.697	
Frontal cortex		
All group	<0.001	<i>F</i> (3,20)=28.354
Vehicle group vs. Vit.E group	<0.001	

Vehicle group vs. AP 250 group	0.999	
Vehicle group vs. AP 500 group	<0.001	
AP 250 group vs. AP 500 group	<0.001	
Vit.E group vs. AP 250 group	<0.001	
Vit.E group vs. AP 500 group	0.971	