

Supplementary information

Standardized phytotherapeutic extracts rescue anomalous locomotion and electrophysiological responses of TDP-43 *Drosophila melanogaster* model of ALS

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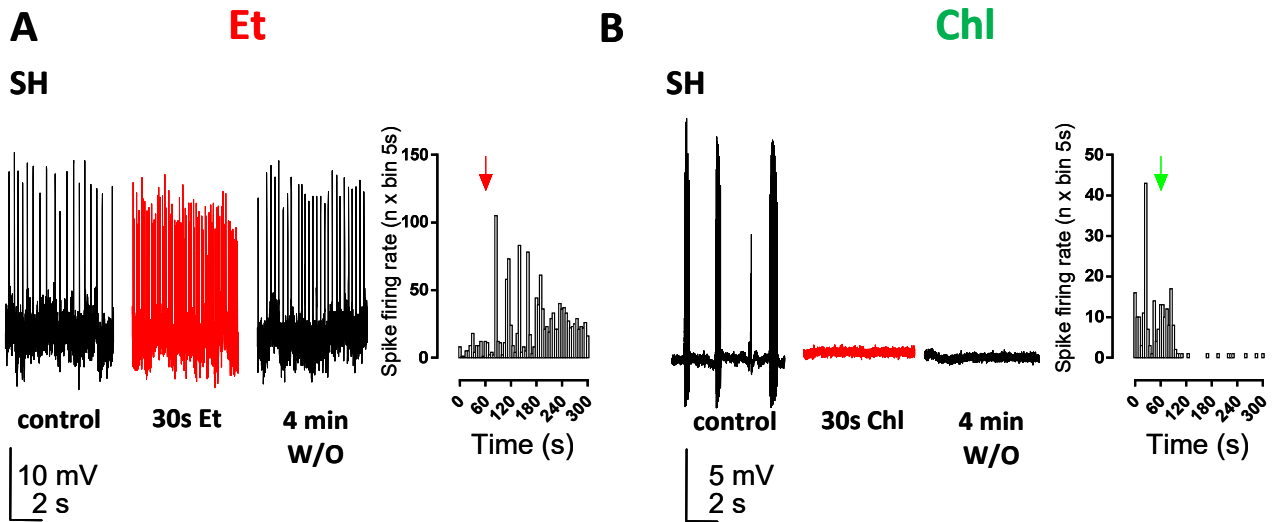
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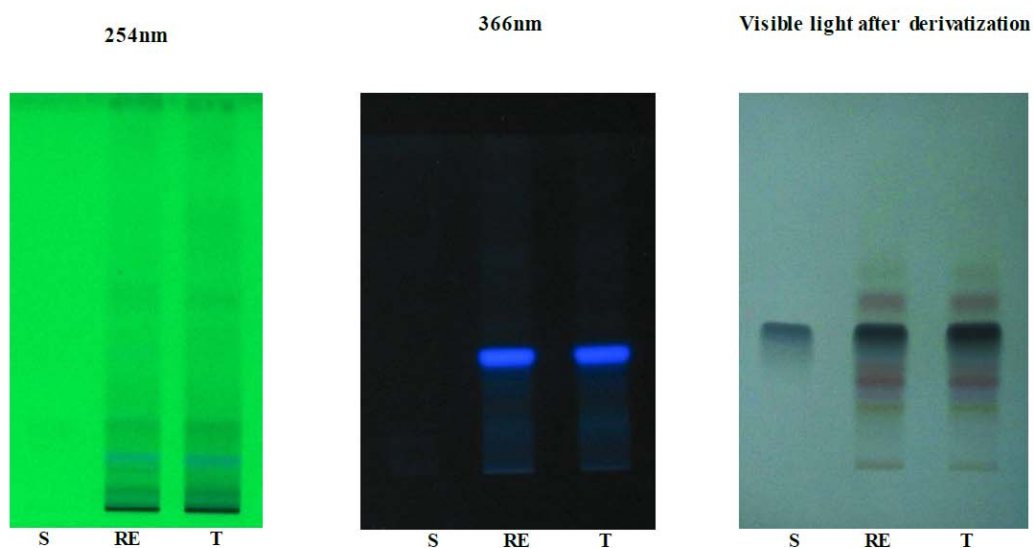
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Supplementary Figure S1: Representative traces and plots obtained from SH mutant flies, in which spontaneous PSPs were recorded in the absence, after 30 s and after 4 min of ether (Et) or chloroform (Chl) exposure. Arrows in graphs indicate when vapours were delivered. Scale bars: left panels, 10 mV/2 s, right panels 5 mV/2 s.

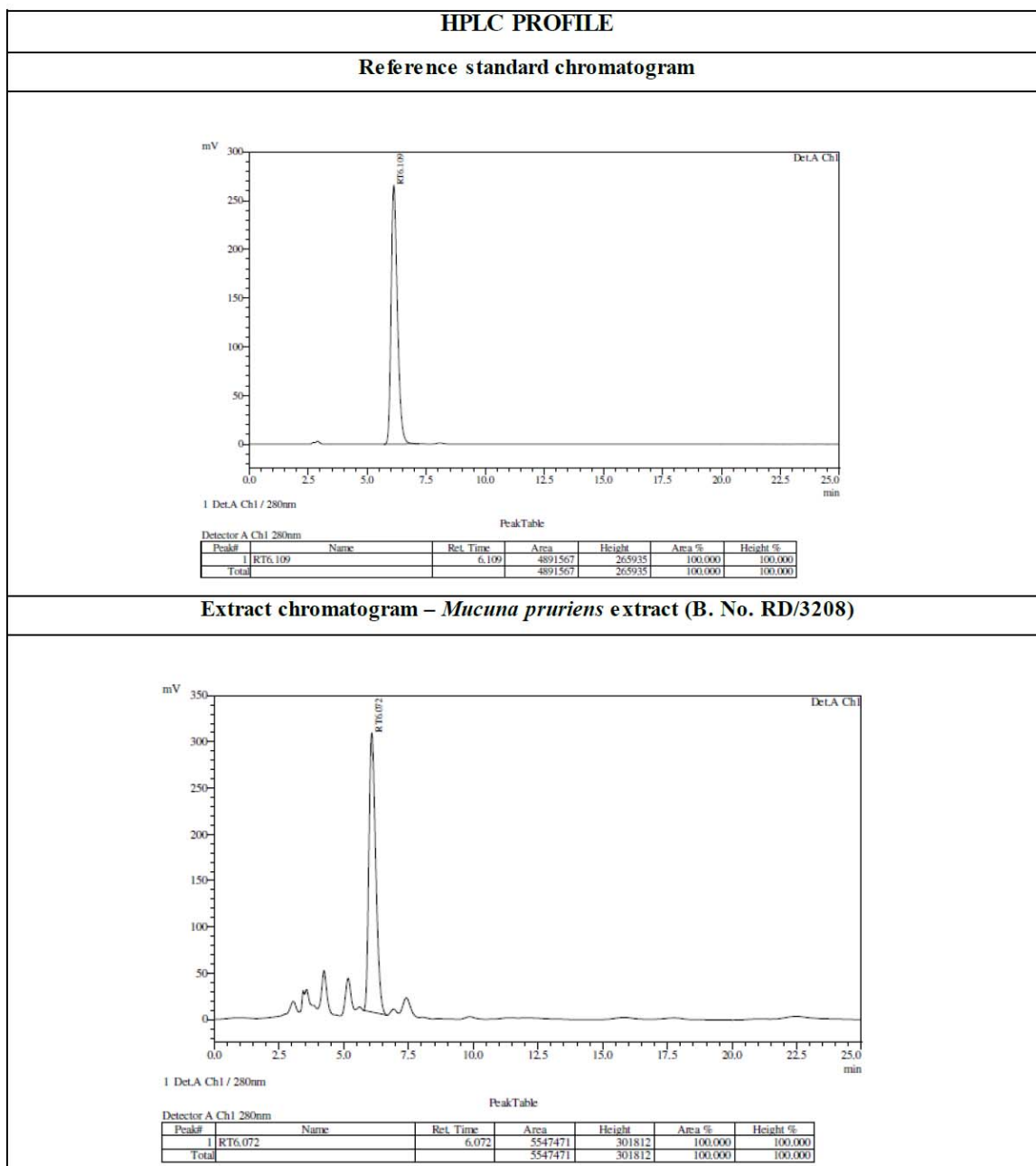
TLC PROFILE

Product Name	:	<i>Mucuna pruriens</i> extract (≥ 10% L-Dopa)
Batch No.	:	RD/3208
Reference extract used for comparison		MP/140101



S : L-Dopa reference standard B. No. 59200
 RE : *Mucuna pruriens* reference extract (prepared using crude drug reference standard (CDRS) B. No. 386) B. No. MP/140101
 T : *Mucuna pruriens* extract B. No. RD/3208

METHOD ADOPTED	
SOP No.	: NR/QCD/APM05 WI(31)
Mobile Phase	: Butanol : Ethanol : Acetic acid : Water (60 : 32 : 12: 8)
Detection	: Ninhydrine (30mg of ninhydrine in 10ml of Butanol & 0.3ml of Glacial acetic acid)
Standard Preparation	: 5 mg of L-Dopa dissolved in 5ml of 30%v/v aqueous Orthophosphoric acid solution diluted with methanol to 10ml
Sample Preparation	: 500mg of extract + 10ml 30%v/v aqueous Orthophosphoric acid solution diluted to 100ml with methanol



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METHOD ADOPTED		
SOP No.	:	NR/QCD/APM06 WI(31)
Mobile phase	:	Buffer : Acetonitrile 92 : 8 (Buffer: 1.36gm of potassium di hydrogen orthophosphate to 900ml of water and adjust the pH to 2.5 using orthophosphoric acid).
Column	:	C18-ODS. 5 μ size, 250 x 4.6 mm (Merck).
Flow Rate	:	2.0 ml/ min
Wave Length	:	280 nm
Standard Preparation	:	500mcg/ml L-Dopa is dissolved in 5ml 30% orthophosphoric acid. Add water and make up to 100ml
Sample Preparation	:	5000mcg/ml of extract is dissolved in 5 ml of 30% orthophosphoric acid. Add water and make up to 100ml.

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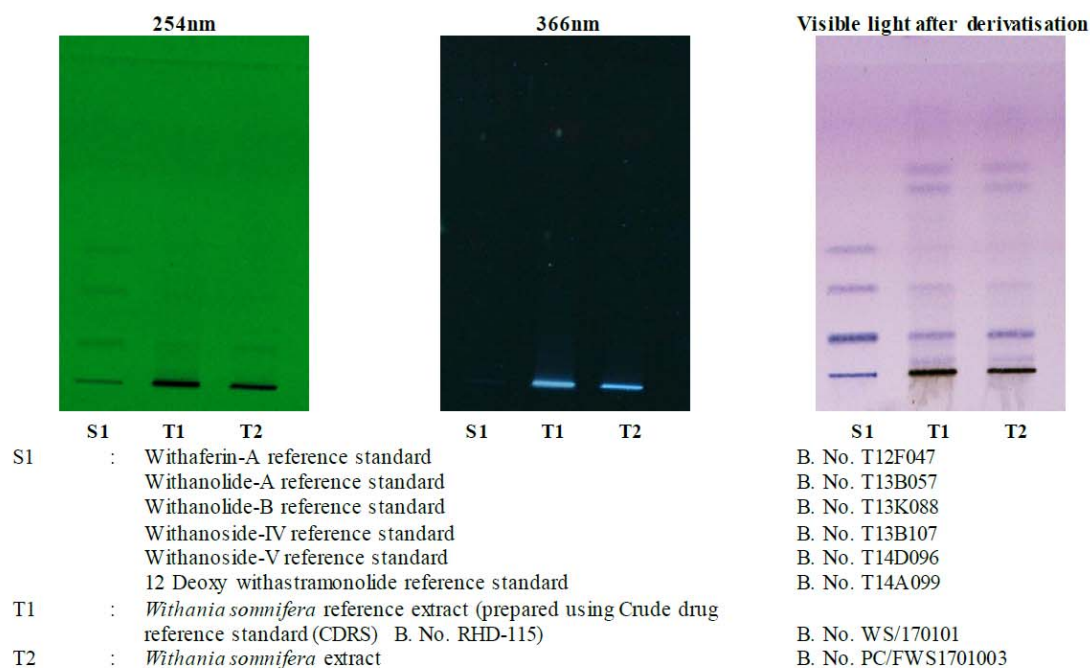
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TLC PROFILE

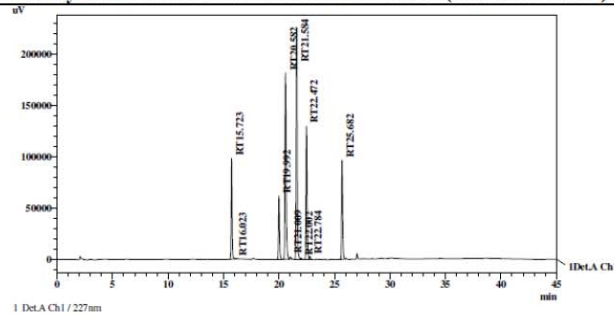
Product Name	:	<i>Withania somnifera</i> extract
Batch No.	:	PC/FWS1701003
Reference extract used for comparison	:	WS/170101
Source of reference standard	:	Natural Remedies Pvt. Ltd.



METHOD ADOPTED	
SOP No.	: NR/QCD/APM05 WI(17)
Mobile Phase	: Ethyl Acetate : Toluene : Acetic acid 45 : 55 : 3
Detection	: Anisaldehyde Sulphuric acid
Standard Preparation (All withanolides)	: 0.5mg of each withanolides per ml in Methanol
Sample Preparation	: 3g of extract in 50ml Methanol

HPLC PROFILE

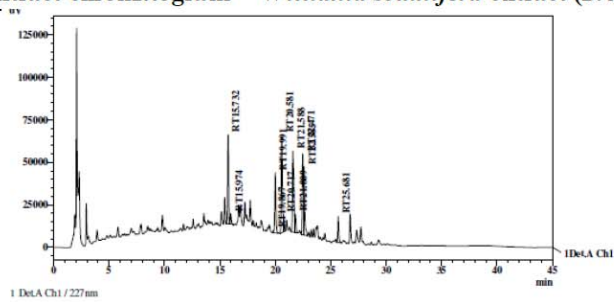
Chromatogram - Standard mix Withaferin-A (B. No. T12F047), Withanolide-A (B. No. T13B057), Withanolide-B (B. No. T13K088), Withanoside IV (B. No. T13B107), Withanoside V (B. No. T14D096), 12-Deoxywithastramonolide reference standard (B. No. T14A099)



PeakTable @E:\2017\Glycyrrhiza\Jan 2017\17.01.2017\Data004.lcd

Peak#	Name	Ret. Time	Area	Area %
1	RT15.723	15.723	597483	11.162
2	RT16.023	16.023	3585	0.067
3	RT19.992	19.992	425917	7.914
4	RT20.582	20.582	1324782	24.616
5	RT21.009	21.009	17610	0.327
6	RT21.584	21.584	1506534	27.988
7	RT22.002	22.002	9026	0.168
8	RT22.472	22.472	855200	15.890
9	RT22.784	22.784	19897	0.370
10	RT25.682	25.682	622501	11.547
Total			5381834	100.000

Reference Extract chromatogram – *Withania somnifera* extract (B. No WS/170101)

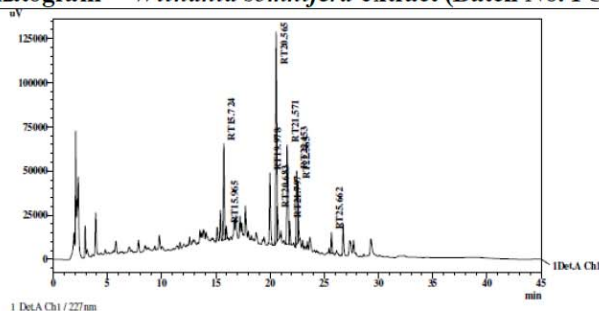


PeakTable @E:\2017\Glycyrrhiza\Jan 2017\17.01.2017\Data006.lcd

Peak#	Name	Ret. Time	Area	Area %
1	RT15.732	15.732	310879	13.994
2	RT15.974	15.974	29238	1.265
3	RT19.867	19.867	2125	0.231
4	RT19.991	19.991	245942	11.071
5	RT20.581	20.581	409097	18.411
6	RT20.717	20.717	51603	2.323
7	RT21.588	21.588	442010	19.896
8	RT21.809	21.809	6554	0.281
9	RT22.471	22.471	278184	12.513
10	RT23.585	23.585	275250	12.490
11	RT25.681	25.681	98850	4.450
Total			2221554	100.000

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Extract chromatogram – *Withania somnifera* extract (Batch No. PC/FWS1701003)



PeakTable: @E:\2017\Withania\Jan 2017\17.01.2017\Data007.Jed

Peak#	Name	Ret. Time	Area	Area %
1	RT15.724	15.724	321897	12.139
2	RT15.965	15.965	41570	1.568
3	RT19.976	19.976	282288	10.355
4	RT20.565	20.565	818719	30.074
5	RT20.683	20.683	73300	2.764
6	RT21.571	21.571	509448	19.211
7	RT21.797	21.797	86427	3.251
8	RT22.453	22.453	255179	9.633
9	RT22.565	22.565	189145	7.133
10	RT25.662	25.662	71019	2.678
Total			2651810	100.000

METHOD ADOPTED

SOP No.	:	NR/QCD/APM04 WI(17)
Mobile phase	:	1) Buffer: 0.01N potassium dihydrogen phosphate in water and 0.5ml orthophosphoric acid. 2) Acetonitrile (Gradient Elution)
Column	:	LichroCART, Lichrospher, C18-5 μ (Merck)
Flow Rate	:	1.500ml/min
Wave Length	:	227nm
Standard Preparation (All withanolides)	:	0.5mg of each withanolides per ml in Methanol
Sample Preparation	:	40mg/ml of extract in Methanol

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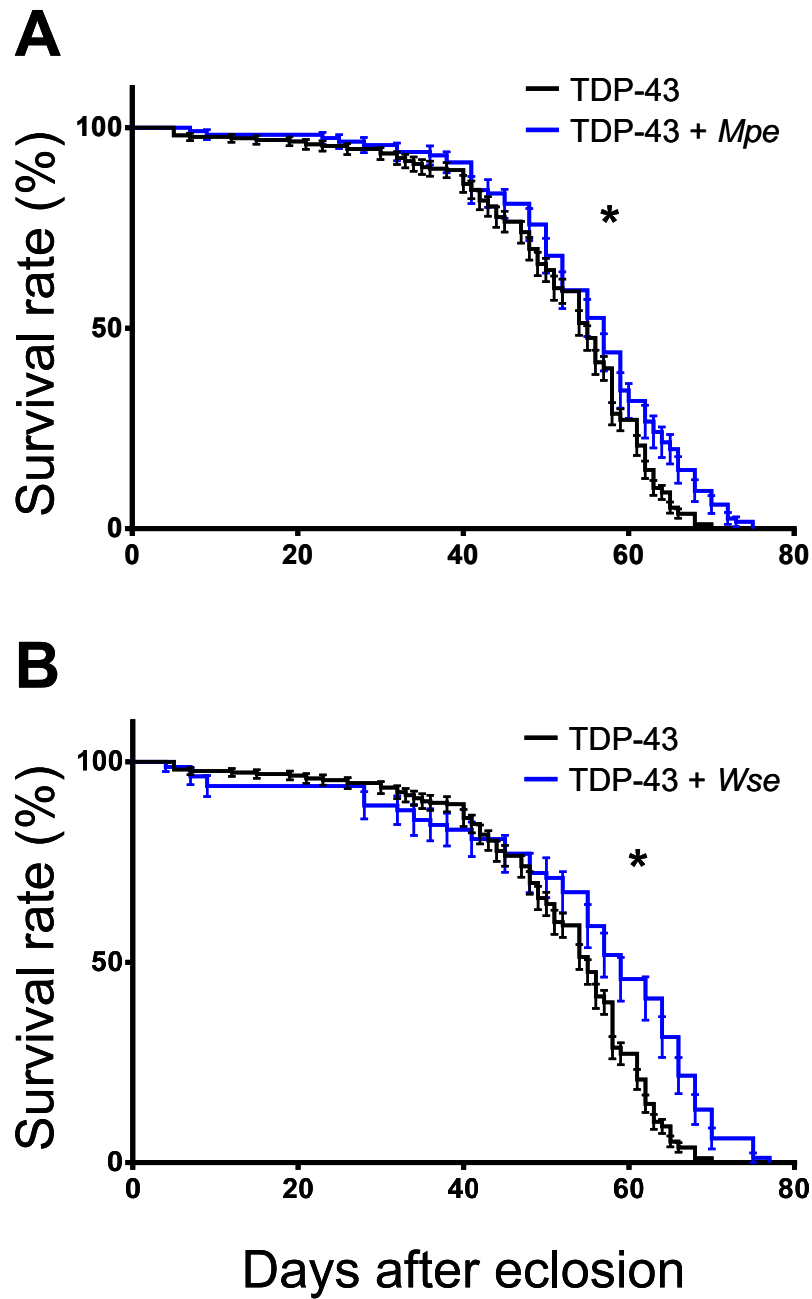
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Supplementary Figure S4: Effects of *Mucuna pruriens* extract (*Mpe*) and *Withania somnifera* extract (*Wse*) treatment on lifespan of TDP-43 mutant. * Indicates $p < 0.05$ at Kaplan-Meier survival curves (Gehan-Breslow-Wilcoxon-Graph Pad Prism 5.01).

Supplementary Video S1: *Drosophila* TDP-43 mutants showing spastic paralysis coupled to shaker-like movements after ether supply.

Supplementary Video S2: *Drosophila* TDP-43 mutants showing spastic paralysis coupled to shaker-like movements after chloroform supply.

Supplementary Video S3: *Drosophila* GAL4 flies do not show any particular movement after ether supply.

Supplementary Video S4: *Drosophila* SH flies do not show any particular movement after chloroform supply.

Supplementary Video S5: *Drosophila* *Wse*-treated TDP-43: treatment prevents the effect of ether administration observed in untreated TDP-43 mutant flies.

Supplementary Video S6: *Drosophila* *Mpe*-treated TDP-43: treatment prevents the effect of ether administration observed in untreated TDP-43 mutant flies.