Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Triphala inhibits alpha-synuclein fibrillization and their interaction study by NMR provides insights into the self-association of the protein

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Materials and methods

Chemical characterization of Triphala

The analysis of the chemical constituents of Triphala was performed by LC-MS with the help of an Agilent 6540 QTOF-MS mass spectrometer and an Agilent 1260 Binary UHPLC liquid chromatography setup. The scan range of the mass spectrometer was 60-1,600 m/z for both negative and positive ion mode. Agilent Zorbax C_{18} column (2.1 x 50 mm, 1.8 μm) was used for the separation of constituents. Mobile phase A was water containing 0.1 % (v/v) formic acid. Mobile phase B was HPLC grade acetonitrile. HPLC was carried out using a linear gradient elution for 30 min with 5 - 95 % B. The instrument settings were as follows: capillary voltage 4.5 kV, nozzle voltage 500 V, fragmentor voltage 150 V, skimmer voltage 45 V, octopole voltage 750 V, nebulizer gas temperature 350 ^{0}C, nebulizer gas pressure 30 psi, nebulizer gas flow 8 l/min, sheath gas temperature 300 ^{0}C, sheath gas flow 10 l/min, cycle time 0.5 s, and run time 30 min. Only the peaks with height greater than or equal to 1000 counts were considered for analysis. The analysis was based on matching of mass spectrum peak with METLIN metabolomics database.[1,2] Mass accuracy was nearly ~10 ppm, whereas mass resolution was found to vary from molecule to molecule within the range of 8,000 - 20,000 ppm.

Preparation of small oligomeric αSyn

Sample consisting of small oligomeric αSyn was prepared by passing the protein solution through a 100 kDa molecular mass cut-off filter. A more detailed discussion of the protocol has been provided elsewhere.[3] The filtrate was used as the starting material for fibrillization experiments.

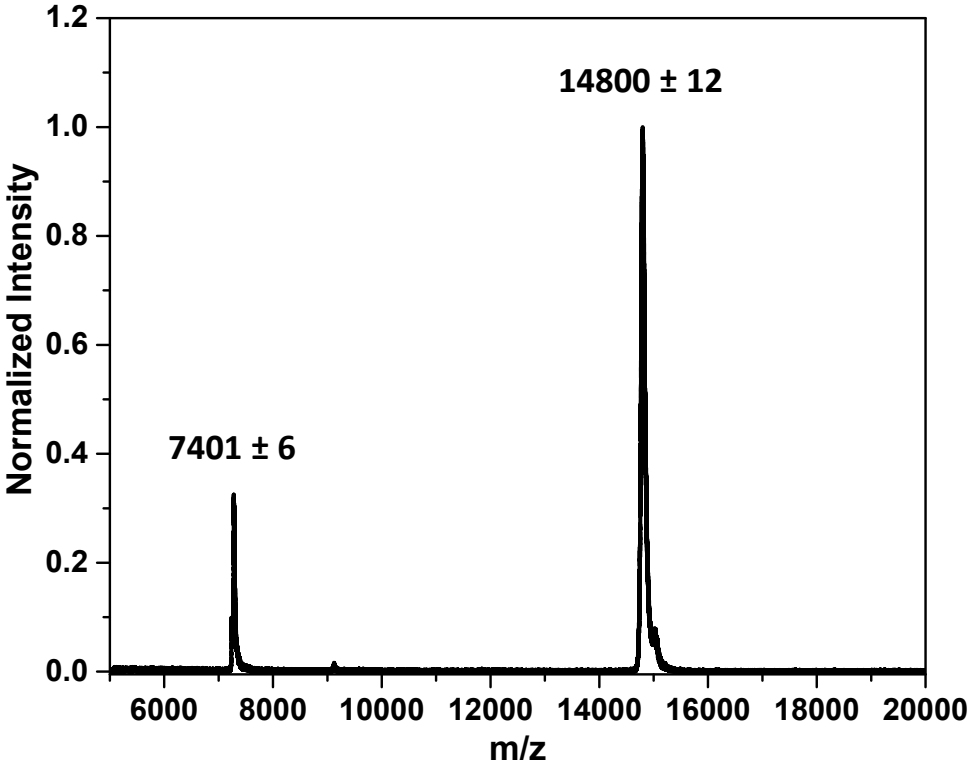


Figure S1. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrum of αSyn. The intense peak at 14,800 (Da) corresponds to the molecular mass of the protein. The less intense peak at 7,401 (Da) is half that of the molecular mass peak and is due to a doubly charged molecular ion. The spectrum indicates that the purification procedure followed has indeed produced pure αSyn protein.

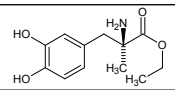
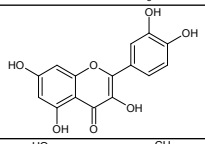
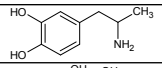
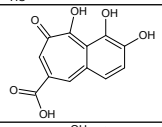
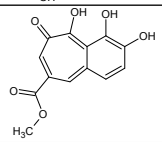
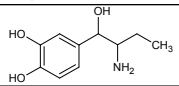
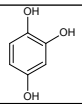
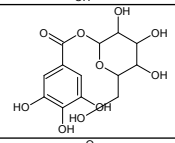
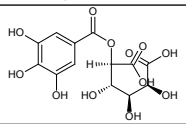
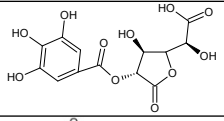
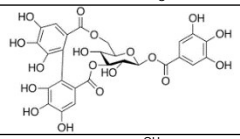
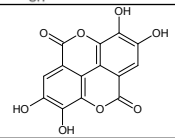
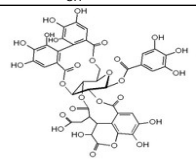
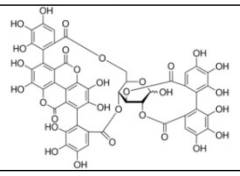
Sr. No.	Compound Name	Compound Structure	Retention Time (min)	m/z	Molecular mass (g/mol)	Peak height	Relative Abundance (%)
1	Methyl dopate		12.743	266.1043	239.1167	88027	0.9427
2	Quercetin		8.682	329.0314	302.0438	14491	0.1552
3	Methyl dopamine		0.777	150.0913	167.0946	93227	0.9984
4	7-Deshydroxypyrogallin-4-carboxylic acid		5.807	247.0257	248.033	83541	0.8947
5	Methyl-7-Deshydroxypyrogallin-4-carboxylic acid		6.999	261.0416	262.049	12856	0.1377
6	Ethylnorepinephrine		0.768	180.1017	197.1051	90476	0.9689
7	Hydroxyhydroquinone		1.184	109.0285	126.0318	59422	0.6364
8	β-Glucogallin		0.683	331.0674	332.0746	186905	2.0016
9	Mucic acid-2-O-gallate		0.775	343.0312	362.0489	90081	0.9647
10	Mucic acid1,4-lactone 2-O-gallate		0.775	343.0312	344.0383	90599	0.9703
11	Corilagin		6.053	635.0908	636.098	36106	0.3867
12	Ellagic acid		6.569	300.9978	302.0051	41523	0.4447
13	Chebulagic acid		6.411	953.0933	954.0994	3542	0.03793
14	Punicalagin		1.016	1083.0608	1084.0676	1591	0.01704

Table S1. Polyphenol constituents of Triphala. The table shows the polyphenol constituents of aqueous extract of Triphala which were detected by LC-MS based chemical characterization. The chemical structures of the constituents were drawn with the help of ChemSketch (ACD Labs, Canada).

Sr. No.	Compound Name	Ion mode	Retention Time (min)	m/z	Molecular mass (g/mol)	Peak height	Relative Abundance (%)
1	Phloionolic acid	Negative	11.462	331.2491	332.2564	94774	1.0150
2	Embelin	Negative	12.466	293.1756	294.1829	275812	2.9538
3	9-Methyl-tridecanoic acid	Negative	17.637	227.2019	228.2091	205133	2.1968
4	Sedoheptulose	Negative	0.477	191.0563	210.0742	141435	1.5147
5	10,11-Epoxy-3,7,11-trimethyl-2E,6E-tridecadienoic acid	Negative	11.288	311.1864	266.1881	52896	0.5665
6	3-Hydroxy-hexadecanoic acid	Negative	18.205	253.2174	272.2353	106159	1.1369
7	Oleandolide	Negative	10.388	431.2285	386.2303	27546	0.2950
8	N-Acetylprimaquine	Negative	11.145	328.1669	301.1791	39959	0.4279
9	3-Hexenedioic acid	Negative	0.789	125.0242	144.0421	510796	5.4703
10	(1R,2R)-3-oxo-2-pentyl-cyclopentanebutanoic acid	Negative	11.586	221.1547	240.1726	50044	0.5359
11	4,12-Dimethyl-tridecanoic acid	Negative	18.267	241.2169	242.2242	27855	0.2983
12	Dodecyl glucoside	Negative	10.556	329.2333	348.2511	28479	0.3050
13	4,12-dihydroxy-hexadecanoic acid	Negative	12.33	287.2233	288.2305	43461	0.4654
14	3-Hydroxyisobutyric acid	Negative	0.492	85.0299	104.0478	198658	2.1275
15	2-Isopropyl-3-methoxycinnamic acid	Negative	11.73	255.0794	220.11	7567	0.08104
16	3-Ethyl-3-methyl-tridecanoic acid	Negative	19.484	255.233	256.2404	998774	10.696
17	Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate	Negative	11.806	309.1701	310.1774	132779	1.4220
18	Quinic acid	Negative	0.518	173.0461	192.0639	38318	0.4104
19	9R-Hydroxy-12E-octadecenoic acid	Negative	18.503	279.2332	298.2511	92895	0.9948
20	1,11-Undecanedicarboxylic acid	Negative	10.991	243.1608	244.1681	45906	0.4916
21	Quinol glucuronide	Negative	0.688	331.0673	286.0692	158374	1.6961
22	Granisetron	Negative	12.671	311.1876	312.1949	218478	2.3398
23	Stearic acid	Negative	21.068	283.2649	284.2722	157757	1.6895
24	N-(2-hydroxyethyl)icosanamide	Negative	17.768	400.343	355.3448	8391	0.08986
25	Nisoldipine	Negative	17.981	387.1558	388.1629	16092	0.1723
26	Argipressin	Negative	10.726	1082.4315	1083.4389	10866	0.1164
27	Mucic acid	Negative	0.501	209.031	210.0383	233991	2.5059
28	3,12-Dihydroxy palmitic acid	Negative	12.558	287.2236	288.2309	14875	0.1593
29	Val Ser Trp	Negative	11.145	371.1726	390.1906	16222	0.1737
30	DL-11-hydroxy stearic acid	Negative	16.077	299.2601	300.2674	74648	0.7994
31	Esmolol	Negative	16.15	276.1614	295.1792	27426	0.2937
32	(1S,2S)-3-oxo-2-pentyl-cyclopentanebutanoic acid	Negative	14.507	221.1555	240.1733	25159	0.2694
33	Syringic acid	Negative	1.123	243.0518	198.0536	31399	0.3363
34	Isopentadecylic acid	Negative	12.671	223.2071	242.2251	20583	0.2204
35	12-oxo-ETE	Negative	16.13	299.2025	318.2203	15948	0.1708
36	Arabinonic acid	Negative	0.494	147.0304	166.0483	73841	0.7908
37	Bendroflumethiazide	Negative	14.005	420.0297	421.0371	4111	0.04403
38	5β-Cholestane-3α,7α,12α,25,26-pentol	Negative	19.476	433.333	452.3507	14217	0.1522
39	a-[1-(diethylamino)ethyl]-p-hydroxy-Benzyl alcohol	Negative	15.829	250.1456	223.158	218460	2.3396
40	Lauric acid	Negative	15.632	199.1712	200.1785	72579	0.7773
41	Salmeterol	Negative	10.314	396.2554	415.2731	16962	0.1816
42	7,8-dihydroxy stearic acid	Negative	13.614	315.2551	316.2624	67540	0.7233
43	2H-1-Benzopyran-6-acetic acid, 7-hydroxy-8-methoxy-2-oxo-	Negative	6.706	277.0361	250.0483	128834	1.3797
44	Punctaporin B	Negative	14.476	233.1557	252.1735	233040	2.4957
45	13-Methyl-hexadecanoic acid	Negative	20.314	269.2494	270.2566	27696	0.2966
46	10-Deoxymethynolide	Negative	13.508	277.1819	296.1998	35339	0.3785
47	5-Hydroxy-hexadecanoic acid	Negative	17.384	271.2288	272.2361	23806	0.2549
48	D-Saccharic acid	Negative	0.776	191.0204	210.038	41486	0.4443
49	Gln Pro His	Negative	11.586	361.1634	380.1813	10301	0.1103

Sr. No.	Compound Name	Ion mode	Retention Time (min)	m/z	Molecular mass (g/mol)	Peak height	Relative Abundance (%)
50	Madecassic acid	Negative	10.355	503.337	504.3443	15402	0.1649
51	1-(9Z-Heptadecenoyl)-2-octadecanoyl-sn-glycerol	Negative	21.071	589.5196	608.5371	5765	0.06174
52	17-Phenyl-trinor-PGE2	Negative	14.47	367.1928	386.2107	60906	0.6523
53	3R-Hydroxy-tetradecanoic acid	Negative	14.661	243.1974	244.2046	7984	0.08550
54	Nalbuphine	Negative	15.565	384.1829	357.1954	32498	0.3480
55	5'-Hydroxy-hydrodolasetron	Negative	17.384	401.1733	342.1593	48528	0.5197
56	Phenyl glucuronide	Negative	1.282	315.0728	270.0747	5751	0.06159
57	19-Hydroxy-nonadecanoic acid	Negative	16.836	313.2758	314.2832	11450	0.1226
58	Docusate	Negative	19.489	421.2264	422.2337	8307	0.08896
59	GPEtn(18:1(9Z)/0:0)[U]	Negative	14.503	478.2954	479.3027	13737	0.1471
60	Dodecanedioic acid	Negative	10.168	229.1455	230.1525	15449	0.1654
61	9-Oxo-nonanoic acid	Negative	10.592	171.1031	172.1103	7185	0.07695
62	Melibiose	Negative	0.477	377.0866	342.1176	14850	0.1590
63	GPEtn(18:0/0:0)	Negative	14.228	540.3323	481.3183	4964	0.05316
64	p-Hydroxynorpropoxyphene	Negative	17.178	386.1987	341.2004	6022	0.06449
65	1-Octadecanoyl-rac-glycerol	Negative	19.772	393.278	358.3083	3398	0.03639
66	Spectinomycin	Negative	11.806	367.129	332.1594	3025	0.03240
67	Idebenone Metabolite (QS-10)	Negative	12.67	333.169	352.1868	6812	0.07295
68	4-(4-Chlorophenyl)-a-(4-fluorophenyl)-4-hydroxy-1-piperidinebutanol	Negative	21.073	418.1599	377.1555	2850	0.03052
69	15-Hydroxy-pentadecanoic acid	Negative	17.275	239.2029	258.2209	12019	0.1287
70	GPEtn(16:0/0:0)	Negative	14.119	452.2798	453.2868	7510	0.08043
71	GPInsP2[3',4'](17:0/20:4(5Z,8Z,11Z,14Z))	Negative	9.897	1077.4753	1032.476	21186	0.2269
72	Pyrocatechol glucuronide	Negative	4.697	267.0518	286.0694	5406	0.05790
73	15(R)-HEDE	Negative	21.07	351.2531	324.2651	5573	0.05968
74	2-Hydroxy hendecanoic acid	Negative	12.281	201.15	202.1573	7212	0.07724
75	Trp Ile Thr	Negative	9.713	417.2134	418.2207	13464	0.1442
76	Dihydrojasmonic acid, Methyl Ester	Negative	11.486	207.1393	226.1571	103198	1.1052
77	3-Dehydroshikimic acid	Negative	1.411	153.0196	172.0374	5145	0.05510
78	Dihydroxyacetone	Negative	0.49	89.0247	90.032	14371	0.1539
79	N-Methyl-D-aspartic acid	Negative	0.608	128.0355	147.0534	11287	0.1209
80	Trp Ile Thr	Negative	9.841	417.2139	418.2214	8048	0.08619
81	Glyceric acid	Negative	0.588	87.0092	106.027	12926	0.1384
82	Glutaconic acid	Negative	0.585	111.0092	130.027	15289	0.1637
83	12-Keto tridecanoic acid	Negative	11.754	209.1543	228.1721	6691	0.07166
84	Diglycolic acid	Negative	0.52	133.0147	134.0219	28101	0.3009
85	Tetradecanedioic acid	Negative	11.786	257.1757	258.183	8697	0.09314
86	Taurallocholic acid	Negative	9.702	514.2837	515.2916	2976	0.03187
87	6,7-Dimethyl-8-(1-D-ribityl)lumazine	Negative	0.509	353.11	326.1226	9442	0.1011
88	11-hexadecenoic acid	Negative	18.008	253.2173	254.2246	17457	0.1870
89	2-Hydroxy-heptadecanoic acid	Negative	19.09	267.2333	286.2511	11328	0.1213
90	Malic acid	Negative	0.527	115.0042	134.022	16491	0.1766
91	Undecanedioic acid	Negative	9.249	215.129	216.1363	8370	0.08964
92	3,11-Dihydroxy myristoic acid	Negative	10.499	259.1913	260.1986	5004	0.05359
93	7-Methyl-nonanoic acid	Negative	13.496	171.1396	172.1469	4938	0.05288
94	Anisodamine	Negative	11.145	286.1453	305.1631	5649	0.06050
95	10-hydroxy-16-oxo-hexadecanoic acid	Negative	12.671	267.197	286.2148	19269	0.2064
96	Chorismic acid	Negative	0.695	271.0458	226.0475	17014	0.1822
97	Fumarylacetoacetic acid	Negative	1.438	181.0146	200.0324	8128	0.08705
98	5-Carboxymethoxy-3,4-dihydrocarbostyril	Negative	3.696	220.0621	221.0694	6430	0.06886
99	Terephthalic acid	Positive	15.853	149.0234	166.0266	411591	4.4079
100	Byssochlamic acid	Positive	13.44	337.1046	332.1259	158943	1.7022

Sr. No.	Compound Name	Ion mode	Retention Time (min)	m/z	Molecular mass (g/mol)	Peak height	Relative Abundance (%)
101	2-Hydroxy-3-(4-methoxyethylphenoxy)-propanoic acid	Positive	16.626	223.0963	240.0996	23497	0.2516
102	Choline	Positive	0.429	104.1071	104.1077	305261	3.2692
103	3-(4-Hydroxyphenyl)propionic acid	Positive	13.44	149.0598	166.063	58235	0.6237
104	Cycloleucine	Positive	0.505	130.0862	129.079	68015	0.7284
105	N-Acetylserine	Positive	0.595	130.0499	147.0533	36756	0.3936
106	Cloperastine	Positive	8.143	294.1848	329.1542	37648	0.4032
107	Picrotin	Positive	7.03	333.094	310.1048	18819	0.2015
108	Anandamide (20:5, n-3)	Positive	16.267	328.263	345.2663	36347	0.3892
109	Butacaine	Positive	7.518	289.227	306.2302	16315	0.1747
110	3-Oxo-dodecanoic acid	Positive	10.511	237.146	214.1568	24453	0.2619
111	Methyl 4-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-butanoate	Positive	15.269	237.1121	254.1155	11483	0.1230
112	Lactone of PGF-MUM	Positive	15.839	301.1404	296.1617	127254	1.3628
113	Acetyl tyrosine ethyl ester	Positive	11.882	252.1227	251.1155	15701	0.1681
114	(9S,13S)-1a,1b-dinor-10,11-dihydro-12-oxo-15-phytoenoic acid	Positive	14.781	249.1845	266.1877	22854	0.2448
115	Apiole	Positive	15.857	205.0854	222.0888	20485	0.2194
116	3α,7α,12α-Trihydroxy-5α-cholan-24-oic acid	Positive	21.841	413.2654	408.2867	72196	0.7732
117	12-Oxo-14,18-dihydroxy-9Z,13E,15Z-octadecatrienoic acid	Positive	17.458	329.1716	324.193	14734	0.1578
118	2H-1-Benzopyran-2-one, 6-(1,2-dihydroxyethyl)-7-hydroxy-8-methoxy-	Positive	6.646	235.0596	252.0631	12937	0.1385
119	C17 Sphinganine	Positive	18.647	270.2784	287.2817	24265	0.2599
120	Dihydrosphingosine	Positive	19.35	284.2941	301.2974	14207	0.1521
121	5-Phenylvaleric acid	Positive	14.925	161.0962	178.0996	26376	0.2825
122	C16 Sphinganine	Positive	10.685	274.2732	273.266	44117	0.4725
123	Thr Arg	Positive	0.518	280.1383	275.1598	17575	0.1882
124	Nafronyl	Positive	10.51	384.2529	383.2457	11329	0.1213
125	Tridemorph	Positive	20.557	298.3095	297.3022	32810	0.3514
126	Artemisinin	Positive	14.845	287.1251	282.1464	7111	0.07616
127	3-Deoxyarabinohexonic acid	Positive	0.45	203.0526	180.0632	20937	0.2242
128	C17 Sphingosine	Positive	11.684	286.2734	285.2663	13876	0.1486
129	Anandamide (20:3, n-3)	Positive	18.77	332.2939	349.2973	12369	0.1325
130	N-(2-hydroxyethyl) docosanamide	Positive	22.614	388.3562	383.3774	7105	0.07609
131	Nicotinamide mononucleotide	Positive	0.59	339.0346	334.056	16651	0.1783
132	3-O-Sulfogalactosylceramide	Positive	16.305	463. 3413	907.6418	7273	0.07789
133	Teasterone	Positive	21.694	453.3331	448.3543	9677	0.1036
134	4-Dimethoxyphenethylamine	Positive	1.057	164.1068	181.11	9248	0.09904
135	Ethacrynic acid M1	Positive	1.636	306.133	323.1364	11650	0.1248
136	Docosanamide	Positive	22.375	340.3562	339.3489	9643	0.1033
137	3α,7α,12α-Trihydroxy-24-methyl-5β-Cholestan-26-oic acid	Positive	25.23	469.3271	464.3486	20028	0.2145
138	Trp Lys Pro	Positive	6.87	430.2433	429.2359	16618	0.1780
139	Arg Pro	Positive	0.699	276.1439	271.1651	14636	0.1567
140	Tyr Arg	Positive	0.839	342.1541	337.1757	16767	0.1796
141	Gentamicin C1a	Positive	6.24	432.2799	449.2832	12904	0.1382
142	1-docosanoyl-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-sn-glycerol	Positive	20.375	747.5899	724.6	2946	0.03155
143	Cyproterone	Positive	10.538	339.1947	374.1639	5526	0.05918
144	6b,11b,16a,17a,21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	Positive	12.843	415.2108	432.2148	6649	0.07121
145	Pergolide	Positive	0.453	337.1707	314.1817	7246	0.07760
146	GPCho(11:0/0:0)	Positive	10.56	426.263	426.2636	8500	0.09103

Sr. No.	Compound Name	Ion mode	Retention Time (min)	m/z	Molecular mass (g/mol)	Peak height	Relative Abundance (%)
147	Netilmicin	Positive	6.481	476.3058	475.2988	14899	0.1596
148	Phenylethylmalonamide	Positive	1.322	189.1022	206.1052	6215	0.06656
149	Crocetin	Positive	18.285	311.1639	328.1671	5562	0.05957
150	L-O-Methylthreonine	Positive	0.47	116.0706	133.0739	122895	1.3161
151	Glu Ile Lys	Positive	5.948	388.2535	388.2305	7911	0.08472
152	4-Amino-pentanoic acid	Positive	0.463	118.0863	117.079	97460	1.0437
153	Ganglioside GM3 (d18:1/16:0)	Positive	8.501	577.3648	1152.716	2085	0.02233
154	N-Hydroxynorcocaine	Positive	5.177	288.123	305.1264	5207	0.05576
155	Arg Trp Val	Positive	12.844	460.2681	459.2611	3967	0.04248
156	2-Pyridylacetic acid	Positive	0.464	138.055	137.0477	62583	0.6702
157	Desmethylβhistine(2-(2-aminoethyl)pyridine)	Positive	0.593	123.0917	122.0844	8225	0.08808
158	5-Keto-n-caprylic acid	Positive	12.447	141.0909	158.0942	6742	0.07220
159	N-Isovalerylglycine	Positive	0.464	160.0968	159.0896	31579	0.3382
160	Cotinine methonium ion	Positive	1.33	196.0968	191.1182	16466	0.1763
161	3-Amino-3-(4-hydroxyphenyl)propanoate	Positive	0.52	182.081	181.0737	86426	0.9256
162	Clofibric acid	Positive	0.805	196.0966	214.0395	44180	0.4731
163	3-Chlorotyrosine	Positive	0.729	180.0655	215.035	10157	0.1088
164	2-Amino-8-oxo-9,10-epoxy-decanoic acid	Positive	0.479	216.1228	215.1156	44606	0.4777
165	1-Hexadecyl-2-O-acetyl-glycerol	Positive	20.374	381.2966	358.3071	12520	0.1341
166	β-vinyl acrylic acid	Positive	1.186	81.0338	98.037	5583	0.05979
167	Lys Lys Lys	Positive	18.954	425.2863	402.2969	8234	0.08818
168	4-Keto-n-caproic acid	Positive	1.91	113.0598	130.063	10991	0.1177
169	m-Chlorobenzoic acid	Positive	15.854	121.0283	155.9978	9587	0.1027
170	Leu His Gly	Positive	0.592	330.1541	325.1748	24645	0.2639
171	4-Aminobutyraldehyde	Positive	0.475	70.0653	87.0686	9466	0.1014
172	Benzoic acid	Positive	13.44	105.0336	122.0369	6662	0.07135
173	(9S,13S)-1a,1b-dihomo-jasmonic acid	Positive	14.269	243.135	238.1564	5581	0.05977
174	Tyr Ser	Positive	1.046	269.1128	268.1056	10595	0.1135
175	Nitrosobenzene	Positive	0.485	108.0445	107.0373	35834	0.3838
176	Desoximetasone	Positive	9.571	359.2027	376.2053	4515	0.04835
177	4-Amino-4-deoxychorismic acid	Positive	0.555	226.0707	225.0635	11060	0.1184

Table S2. Non-polyphenol constituents of Triphala. The table shows the non-polyphenol constituents of aqueous extract of Triphala which were detected by LC-MS based chemical characterization.

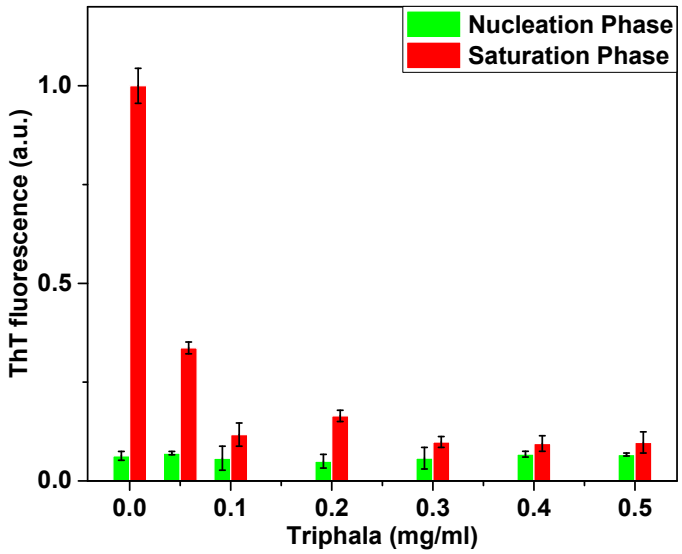


Figure S2. Effect of Triphala on the ThT fluorescence intensity measured in the saturation phase of αSyn fibrillization. The bar chart shows variation in the ThT fluorescence intensity measured in the saturation phase (red) and lag phase (green) of αSyn fibrillization as a function of Triphala concentration. It is clear that the saturation intensity decreases with increase in Triphala concentration, indicating that Triphala is capable of arresting αSyn fibrillization. On the other hand, the lag intensity does not vary with Triphala concentration as expected. The errors are standard deviation (SD).

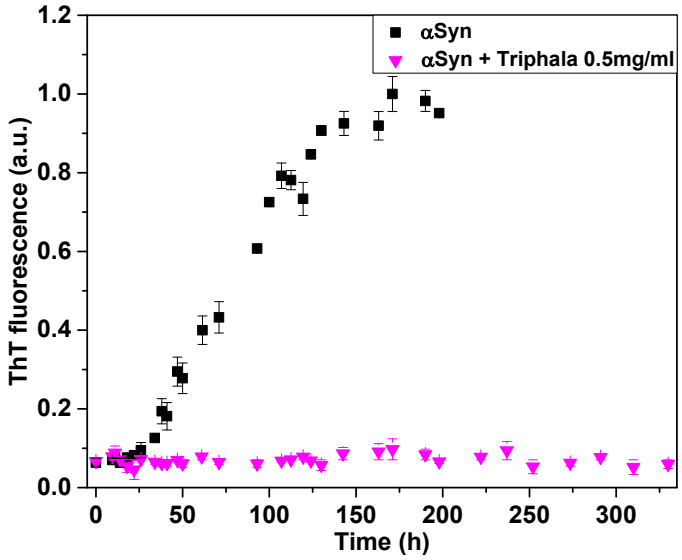


Figure S3. Effect of 0.5 mg/ml Triphala on αSyn fibrillization. The effect of 0.5 mg/ml Triphala on the fibrillization of 150 μM αSyn was studied for an extended duration up to 330 h (in order to investigate if the process of fibrillization initiated beyond 198 h shown in Fig. 1A). However, there was no detectable increase in ThT fluorescence, suggesting that 0.5 mg/ml Triphala inhibited the fibrillization of 150 μM αSyn. The errors are SD.

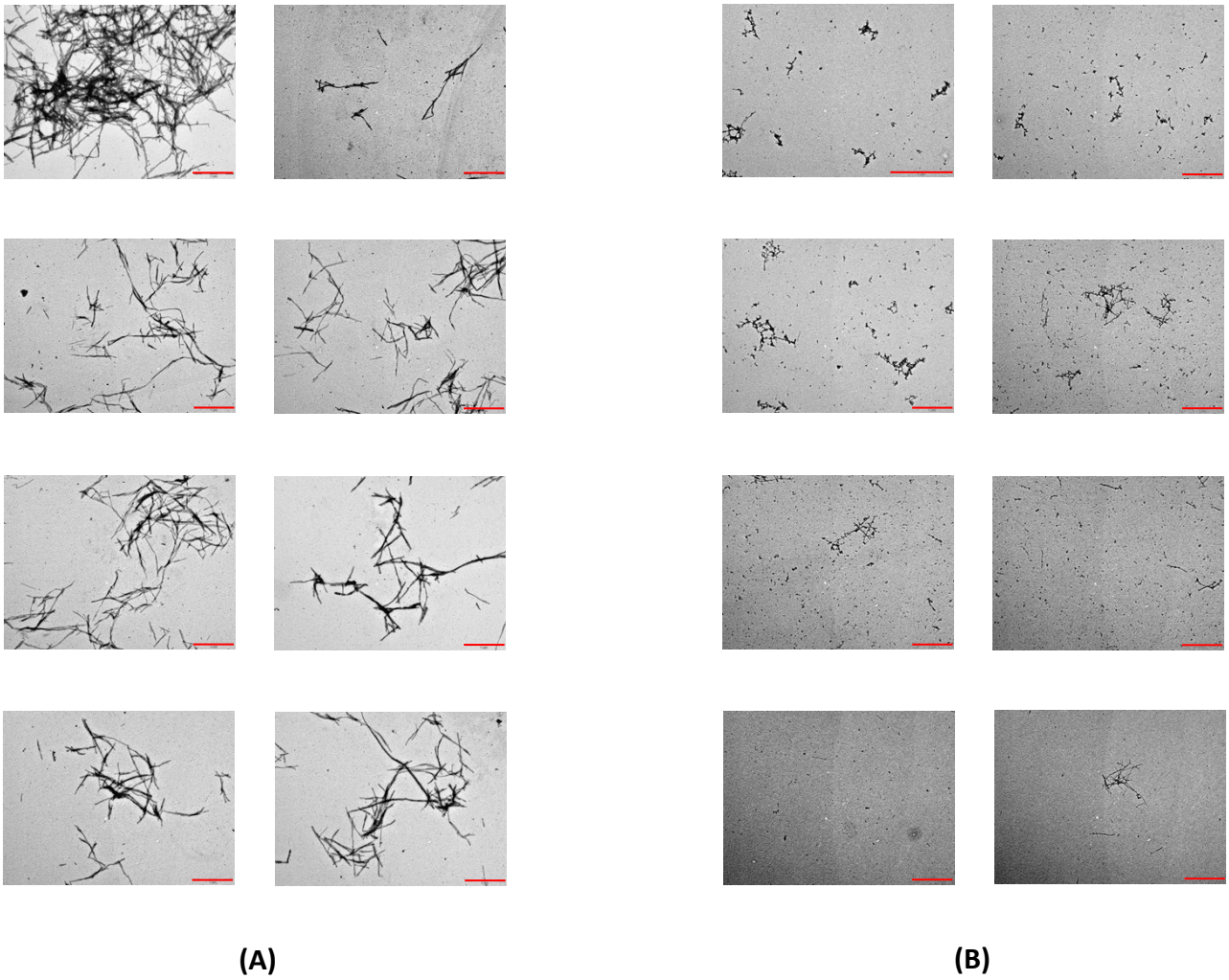


Figure S4. Morphological characterization of the effect of Triphala on αSyn fibrillization. TEM images for αSyn fibrils formed in the absence (A) and presence (B) of 0.5 mg/ml Triphala. These images were recorded from different regions of the same TEM grid for both samples. A significant decrease in the density of fibrils in the presence of Triphala is clearly evident. Also the average length of the fibrils has reduced in the presence of Triphala. The scale bar is 1 μm in each panel.

	αSyn without Triphala				αSyn with Triphala			
Time (hr)	**Parallel β-sheet (%)**	**Anti-parallel β-sheet (%)**	**β Turn (%)**	**Random coil (%)**	**Parallel β-sheet (%)**	**Anti-parallel β-sheet (%)**	**β Turn (%)**	**Random coil (%)**
0	**0**	**23.8 ± 1.8**	**17.4 ± 0.9**	**58.8 ± 2.9**	**0**	**25.3 ± 2.9**	**17.6 ± 0.2**	**57.1 ± 2.5**
16.5	**0**	**25.2 ± 0.4**	**18.2 ± 1.6**	**56.7 ± 3.6**	**0**	**23.3 ± 0.9**	**18.1 ± 0.5**	**58.6 ± 1.2**
24	**0**	**24.8 ± 0.8**	**17.6 ± 1.4**	**57.6 ± 2.8**	**0**	**22.9 ± 0.5**	**17.9 ± 0.3**	**59.2 ± 0.8**
37	**4.3 ± 0.4**	**31.2 ± 2.6**	**19.3 ± 1.9**	**45.3 ± 2.2**	**0**	**21.2 ± 1.2**	**17.8 ± 0.2**	**60.9 ± 1.3**
43	**11.9 ± 1.1**	**28.9 ± 3.3**	**16.5 ± 2.0**	**42.8 ± 2.7**	**0**	**22.2 ± 0.4**	**18.3 ± 0.9**	**59.5 ± 0.4**
49	**15.0 ± 1.2**	**25.1 ± 0.5**	**16.5 ± 1.2**	**43.4 ± 3.7**	**0**	**21.4 ± 1.0**	**17.9 ± 0.7**	**60.7 ± 1.1**
65	**15.9 ± 1.4**	**31.3 ± 3.7**	**14.1 ± 0.7**	**38.8 ± 3.4**	**0**	**22.7 ± 0.7**	**18.3 ± 0.5**	**59.0 ± 1.3**
89	**19.4 ± 0.9**	**26.5 ± 0.9**	**13.2 ± 0.9**	**40.9 ± 3.1**	**0**	**22.0 ± 0.9**	**18.1 ± 0.3**	**59.9 ± 0.9**
120	**22.2 ± 1.6**	**25.3 ± 0.8**	**11.6 ± 0.8**	**41.0 ± 4.1**	**0**	**22.9 ± 1.2**	**17.9 ± 0.4**	**59.2 ± 1.6**
154	**24.3 ± 1.2**	**25.4 ± 0.4**	**10.4 ± 0.7**	**39.8 ± 2.0**	**0**	**22.1 ± 0.6**	**17.8 ± 0.2**	**60.2 ± 1.8**
221	**28.3 ± 1.4**	**20.6 ± 2.0**	**9.2 ± 0.4**	**42.0 ± 3.2**	**0**	**22.6 ± 0.8**	**17.8 ± 0.6**	**59.6 ± 0.5**
265	**29.8 ± 0.8**	**22.8 ± 1.8**	**8.2 ± 0.5**	**39.2 ± 2.6**	**0**	**22.7 ± 1.1**	**18.2 ± 0.8**	**59.0 ± 1.5**
527	**34.4 ± 2.1**	**21.5 ± 1.3**	**5.3 ± 0.6**	**38.8 ± 2.4**	**0**	**20.5 ± 1.9**	**17.8 ± 0.4**	**61.7 ± 2.1**

Table S3. Effect of Triphala on the secondary structure of αSyn. CD spectra of 150 μM αSyn were recorded at various time points after incubation under fibrillating conditions in the absence and presence of 0.75 mg/ml Triphala. Each spectrum was deconvoluted into its constituent secondary structural elements with the help of BESTSEL algorithm.[4,5] The percentage of contribution of each of the secondary structural element obtained is mentioned alongside the time of incubation under fibrillating conditions.

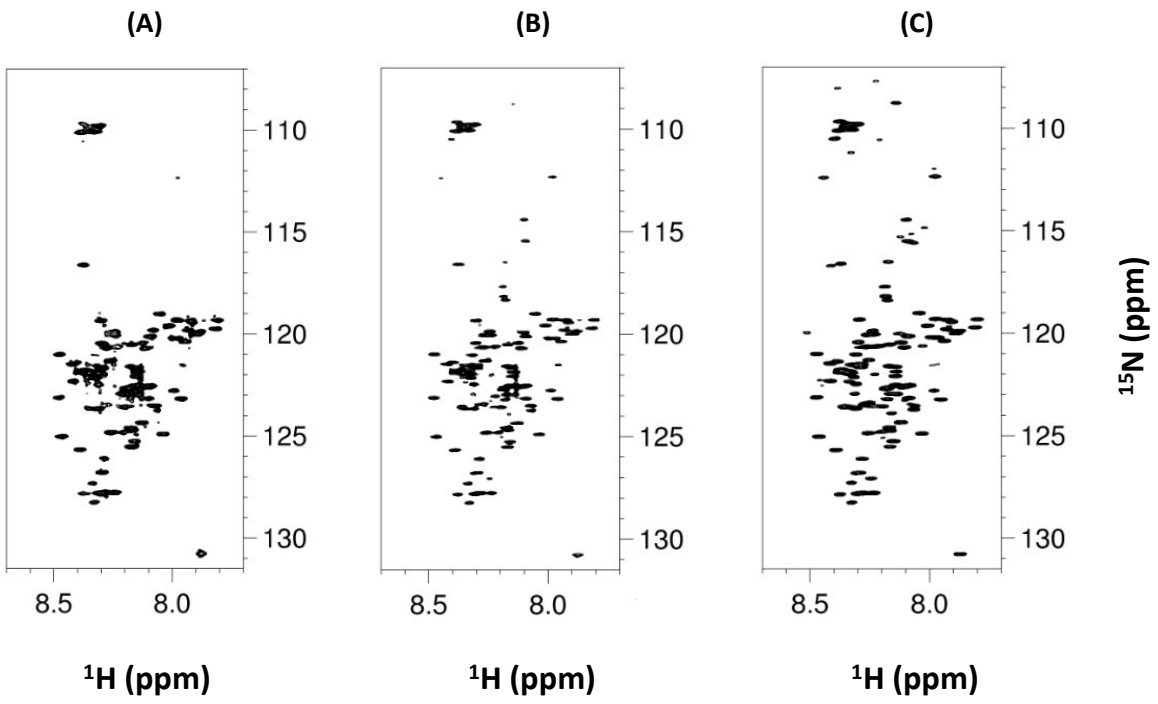


Figure S5. Effect of Triphala on αSyn conformation. ^{1}H-^{15}N HSQC spectrum of αSyn in the absence (A), presence of 0.5 mg/ml (B) and 0.75 mg/ml (C) concentration of Triphala. Systematic appearance of several peaks indicates the shift in monomer-oligomer exchange towards the monomer in the presence of Triphala.

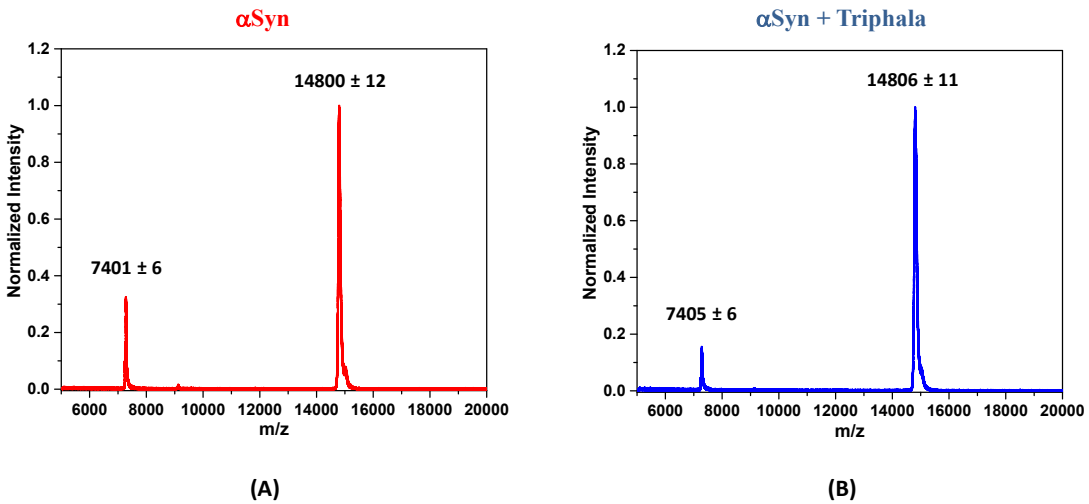


Figure S6. Effect of Triphala on αSyn. MALDI-TOF mass spectrum of αSyn without (A) and with Triphala (B), after overnight incubation under fibrillating conditions.

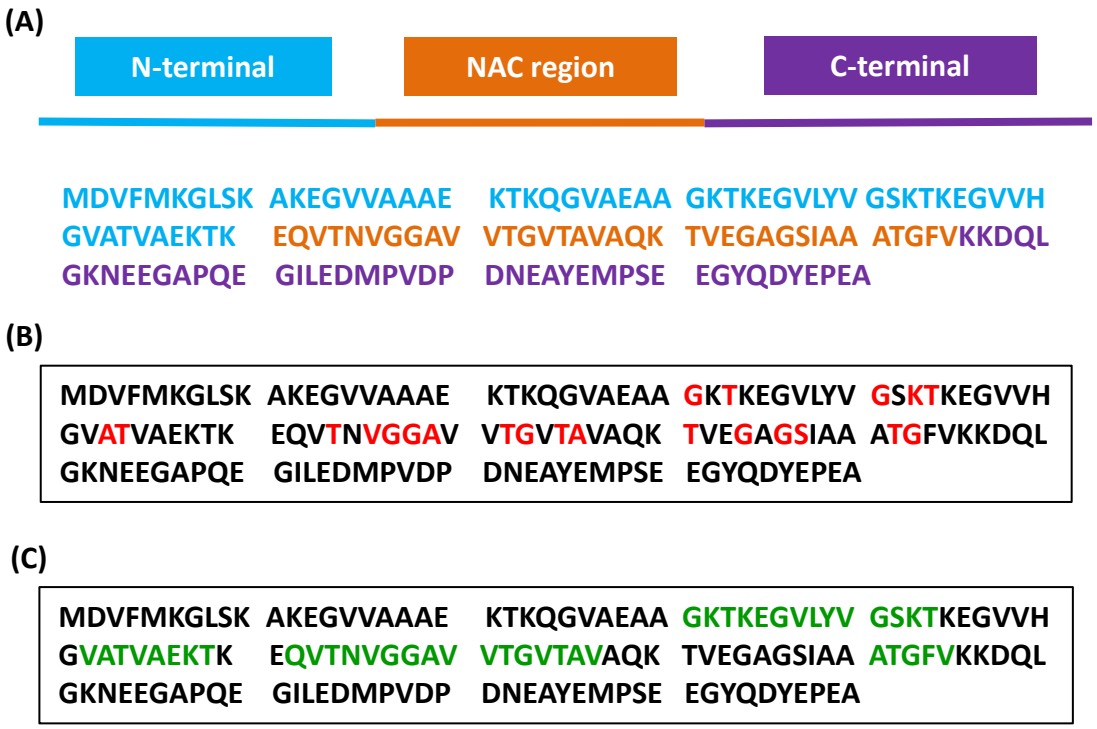


Figure S7. Correlation between the residues for which HSQC peaks vanished and those which constitute the fibril core. (A) The primary sequence of αSyn showing the different regions: N-terminal, NAC and C-terminal regions. (B) The primary sequence of αSyn (black) showing the residues which are responsible for αSyn oligomer formation (red) as revealed by HSQC experiments in the current study. (C) The residues of αSyn which participate in the formation of fibril core (green) as reported previously by Vilar *et. al.*[6]

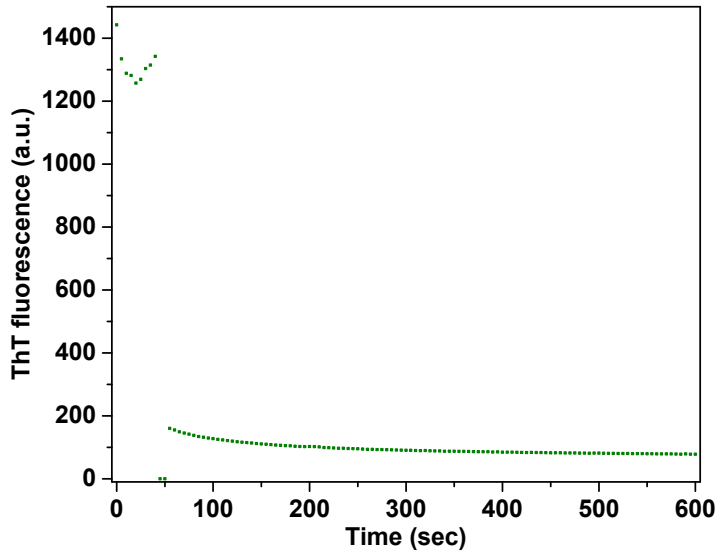
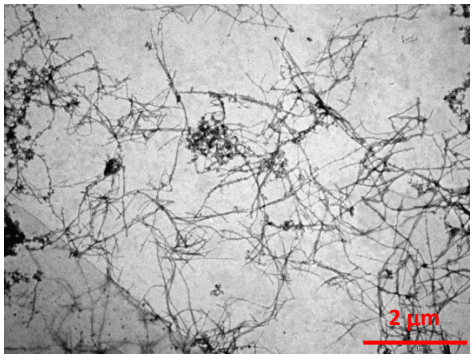
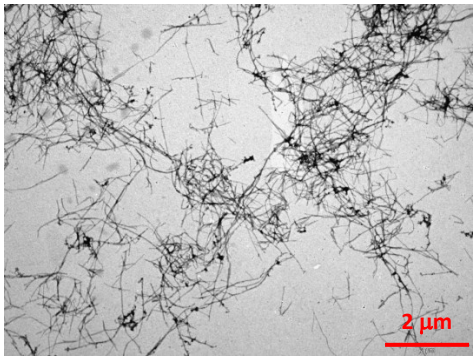


Figure S8. Early effect of Triphala on preformed αSyn fibrils. ThT was added to preformed αSyn fibrils in a 1:5 molar excess, where 3 μM αSyn and 25 μM ThT were the respective concentrations. 0.5 mg/ml Triphala stock solution was added to the sample during time-course ThT fluorescence measurement so that the resulting Triphala concentration was 10 μg/ml. An immediate drop in ThT fluorescence was observed with the addition of Triphala. This indicates that Triphala caused immediate destabilization of αSyn fibrils. Fluorescence measurement was performed with a time interval of 5 sec.



(A)



(B)

Figure S9. Morphological characterization of the effect of 0.5 mg/ml Triphala on preformed αSyn fibrils. 150 µM of small oligomeric αSyn was allowed to fibrillize in PBS pH 7.4. Fibrillized αSyn was divided into two parts. One part was diluted with (A) PBS to obtain a final concentration of 135 µM and the other part was diluted with (B) PBS containing Triphala to obtain a final concentration of 135 µM αSyn and 0.5 mg/ml Triphala. (A) TEM image of fibrillar αSyn without treatment with Triphala. (B) TEM image of prefibrillized αSyn after treatment with 0.5 mg/ml Triphala for a period of 5.5 days.

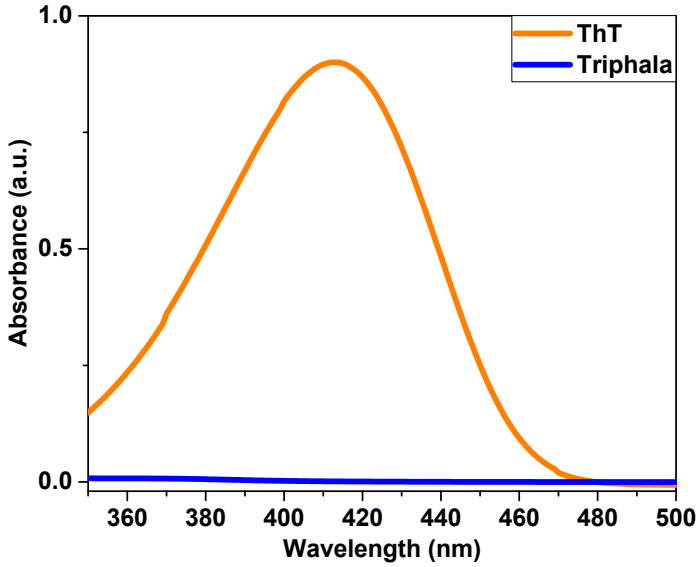


Figure S10. Comparison between the electronic absorption profile of ThT and Triphala. The UV-Vis absorption of ThT (orange) and Triphala (blue) in the wavelength range of 350 - 500 nm is shown.

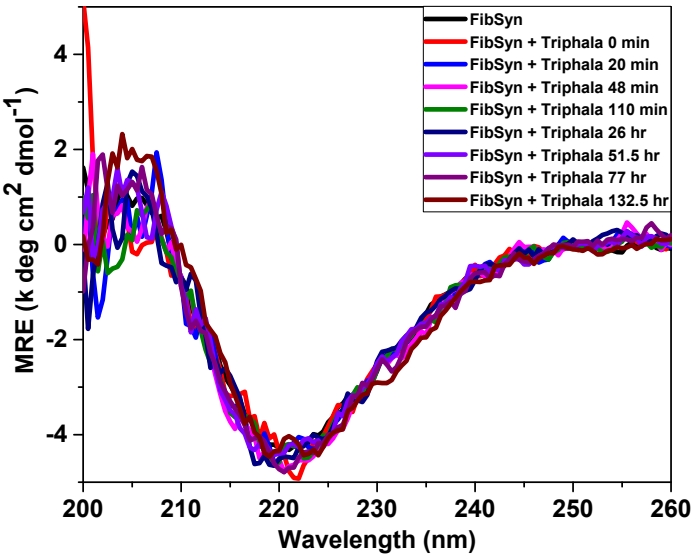
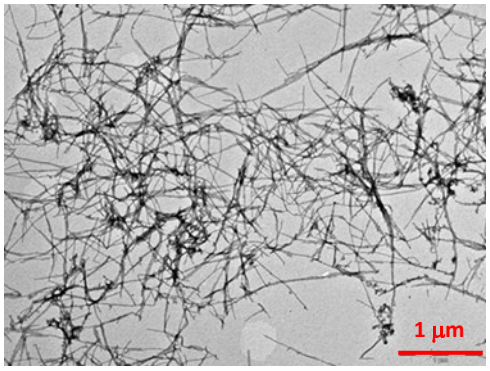
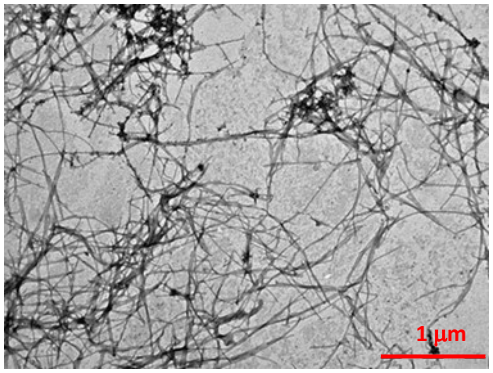


Figure S11. Effect of Triphala on the secondary structure of preformed αSyn fibrils. The changes in the secondary structure of preformed αSyn fibrils upon treatment with 2.5 mg/ml Triphala were studied using CD spectroscopy. Fibriller αSyn showed a minima at ~220 nm which is characteristic of β-sheet. The same feature was observed even after treating the fibrils with 2.5 mg/ml Triphala for 5.5 days. This confirmed that Triphala did not affect the secondary structure of preformed αSyn fibrils. 180 μM of small oligomeric αSyn was allowed to fibrillize in PBS pH 7.4. The CD spectrum of fibrillized αSyn was acquired by diluting 15 μl of protein sample to 255 μl with PBS and was baseline corrected with the spectrum of PBS obtained under the same conditions. Fibrillized αSyn was incubated with Triphala, prepared in the solvent, such that their final concentrations were 135 μM and 2.5 mg/ml respectively. 20 μl aliquots of this sample were withdrawn at various time points and diluted to 255 μl with PBS to acquire αSyn CD spectrum in a temporal manner. Each of these spectra was baseline corrected with PBS containing the appropriate amount of Triphala. Each spectrum was an average of 3 measurements. The bandwidth and data pitch were 1 nm and 0.5 nm respectively.



(A)



(B)

Figure S12. Morphological characterization of the effect of 2.5 mg/ml Triphala on preformed αSyn fibrils. 180 µM of small oligomeric αSyn was allowed to fibrillize in PBS pH 7.4. Fibrillized αSyn was divided into two parts. One part was diluted with (A) PBS to obtain a final concentration of 135 µM and the other part was diluted with (B) PBS containing Triphala to obtain a final concentration of 135 µM αSyn and 2.5 mg/ml Triphala. (A) TEM image of fibriller αSyn without treatment with Triphala. (B) TEM image of prefibrillized αSyn after treatment with 2.5 mg/ml Triphala for a period of 5.5 days.

Supplementary References

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