Supplementary Information for:

Dual surface functionalised curcumin-shellac nano-delivery system with enhanced microbial action

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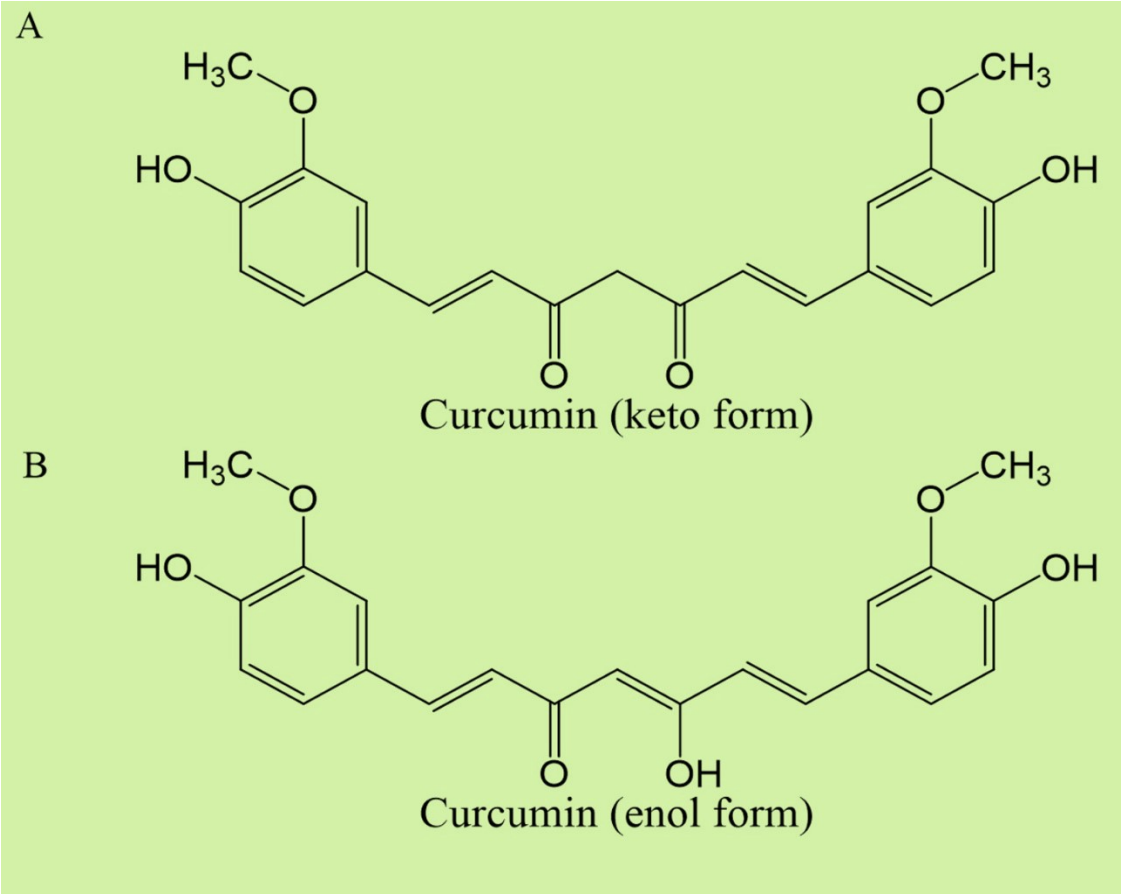


Figure S1: The chemical structure of curcumin (CUR) in: (A) in acidic and natural medium, (B) in alkaline medium.

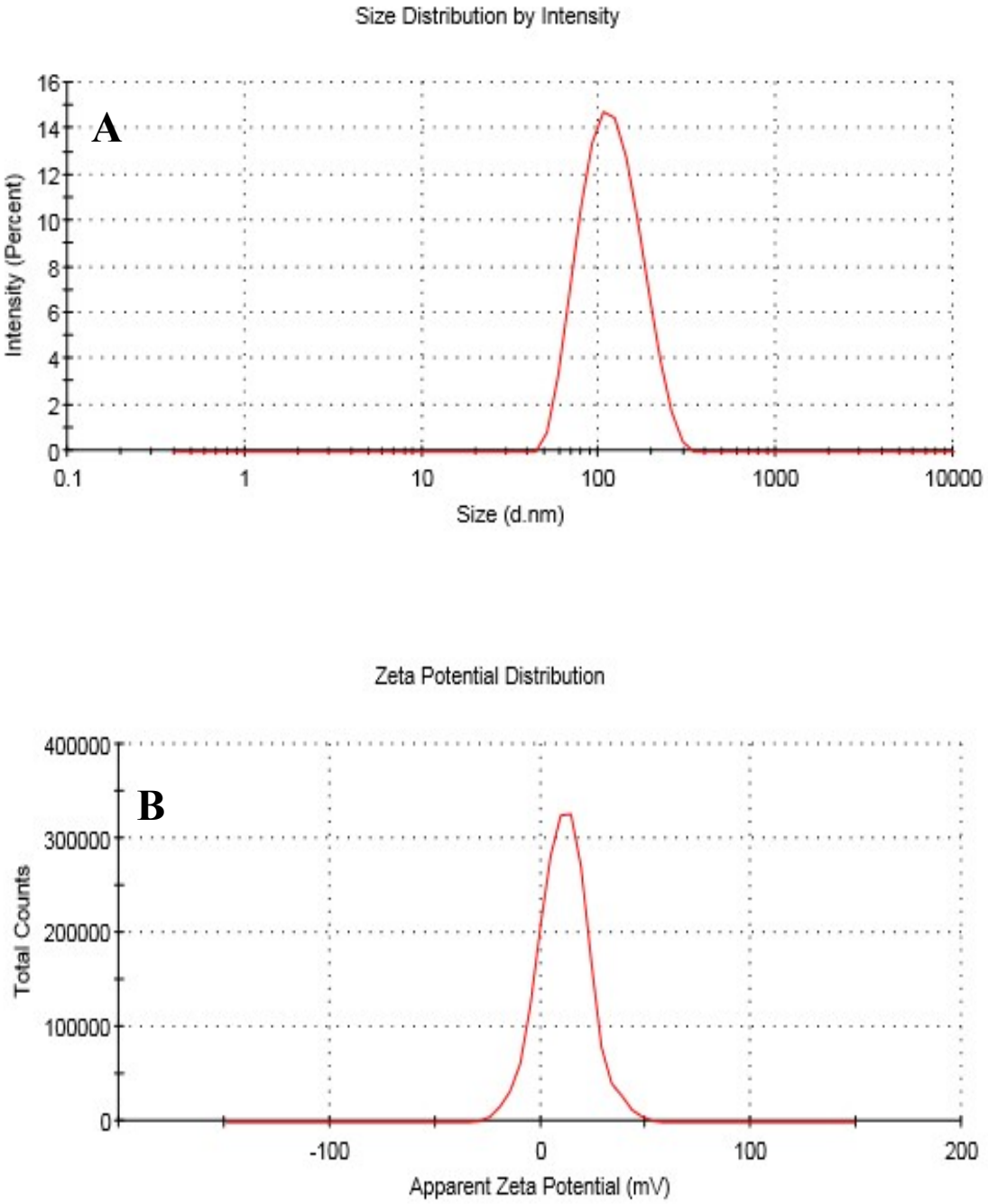


Figure S2: Average Particle size (A) and zeta potential (B) of 0.25 wt. % shellac NPs coated with 0.05 wt. % ODTAB.

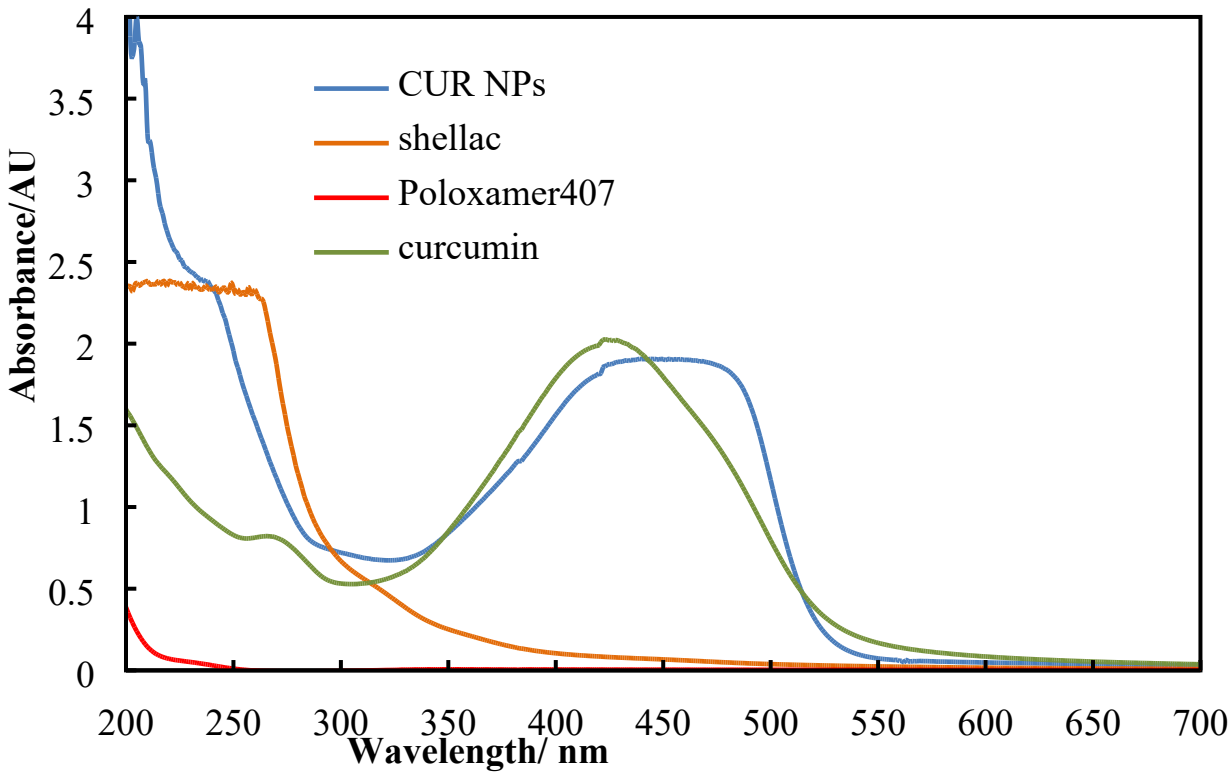


Figure S3. The absorption spectrum of curcumin, free shellac, curcumin loaded shellac NPs and Poloxamer 407 using UV-Vis spectrophotometry technique at range (700-200) nm.

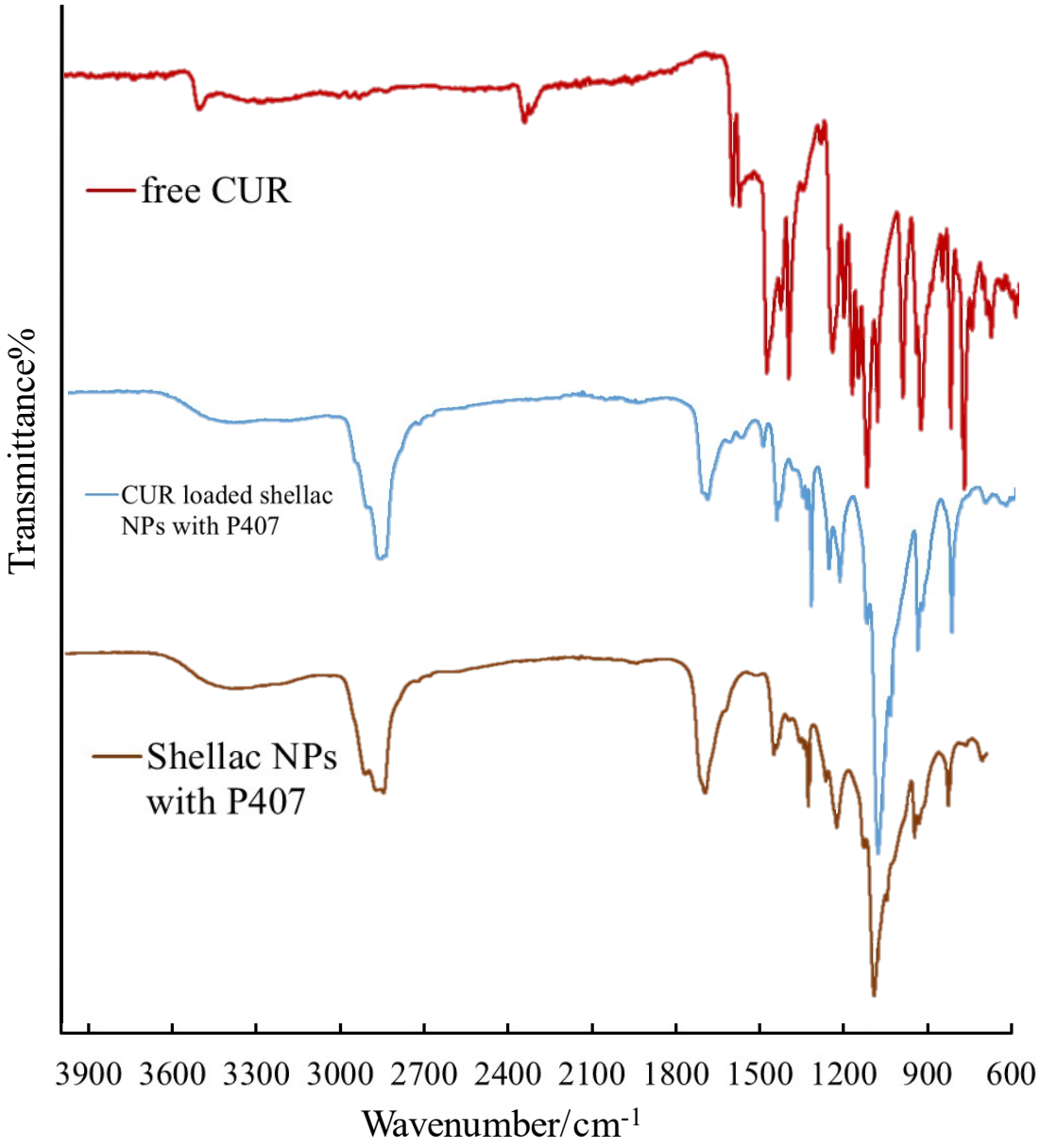


Figure S4. The Fourier Transform-IR spectrum of free curcumin, 0.03 wt.% CUR-NPs, and Shellac NPs with P407 at a range of wavenumber 600-4000 cm^{-1}

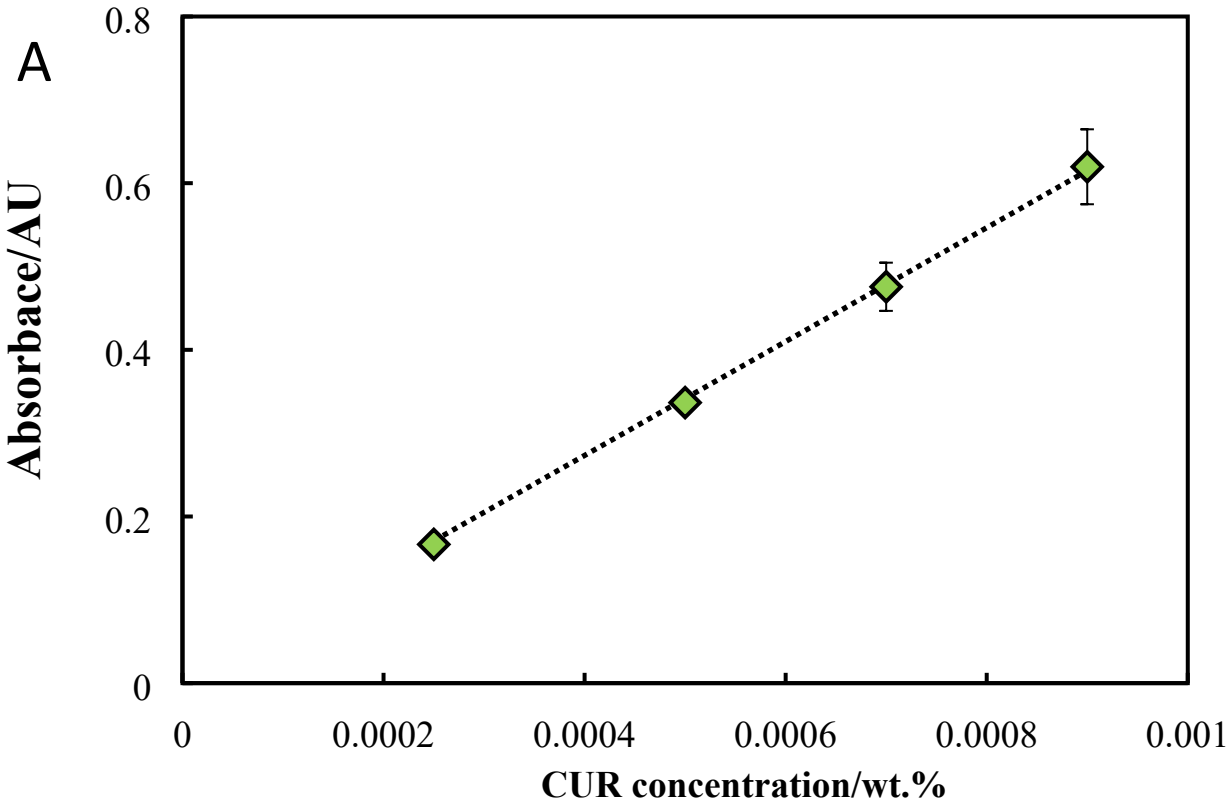
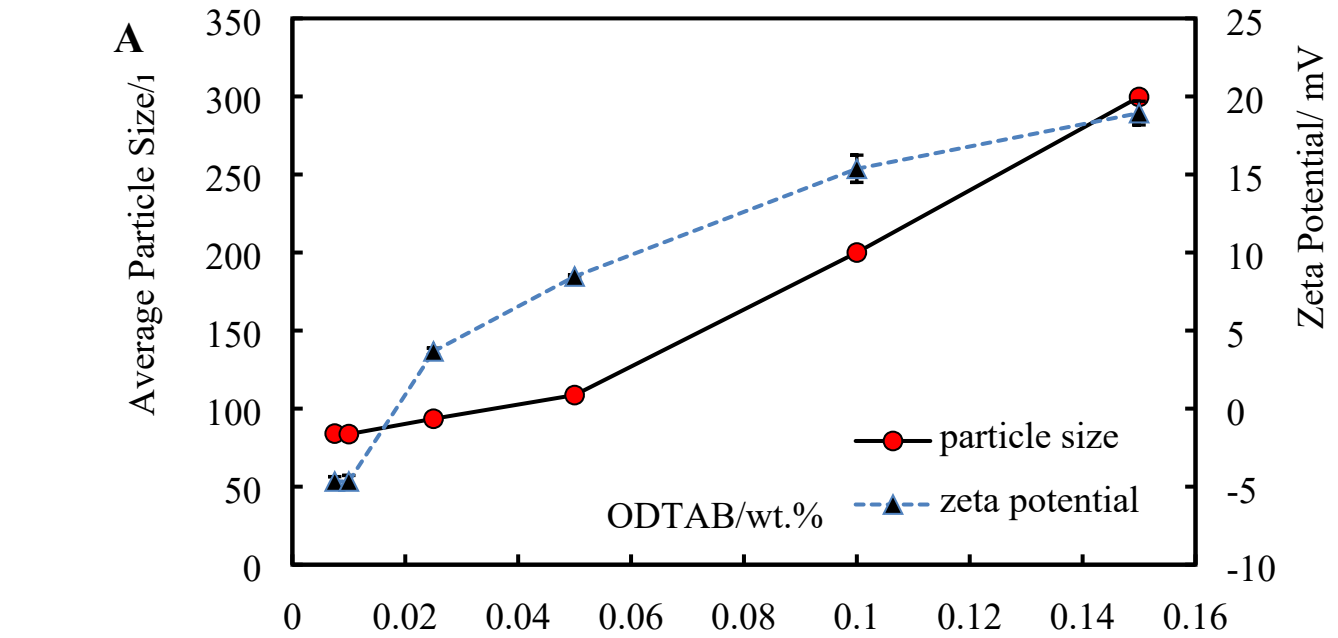


Figure S5. The calibration curve of varies concentrations of free CUR at 426 nm in DI water using UV-Vis spectrophotometer, (n=3).



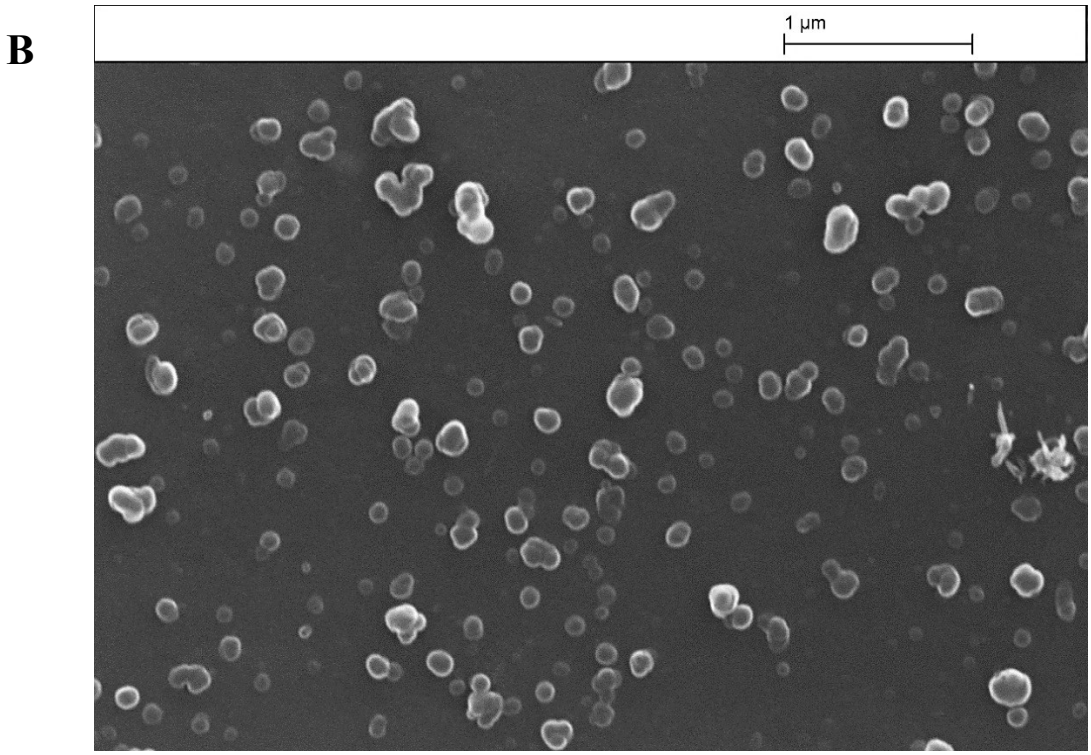
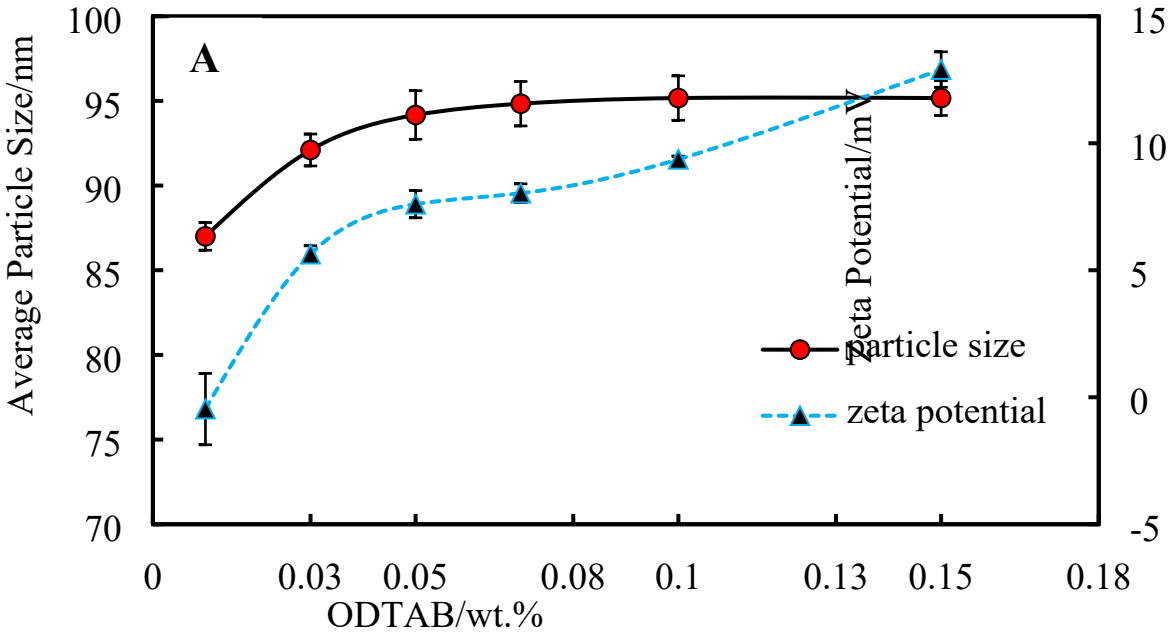


Figure S6: (A) The effect of coating Shellac NPs with different concentrations of ODTAB on the size and zeta potential at pH 5, (n=3). (A) Scanning electron microscopy picture of shellac NPs coated with 0.05 wt.% ODTAB. The NPs was coated with carbon.



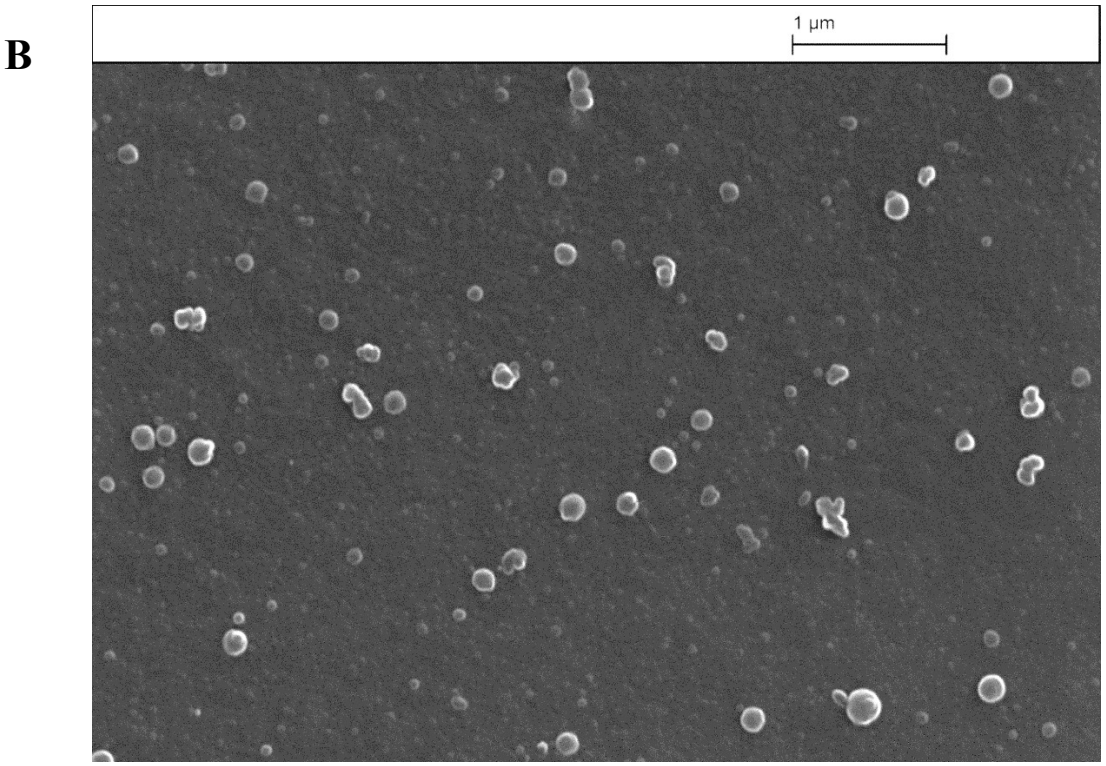


Figure S7: (A) The effect of coating 0.03 wt. % of CUR-loaded Shellac NPs with different concentrations of ODTAB on the size and zeta potential at pH 5, (n=3). (B) Scanning electron microscopy picture of 0.01 wt. % of CUR-loaded shellac NPs after coating with 0.05 wt. % of ODTAB.

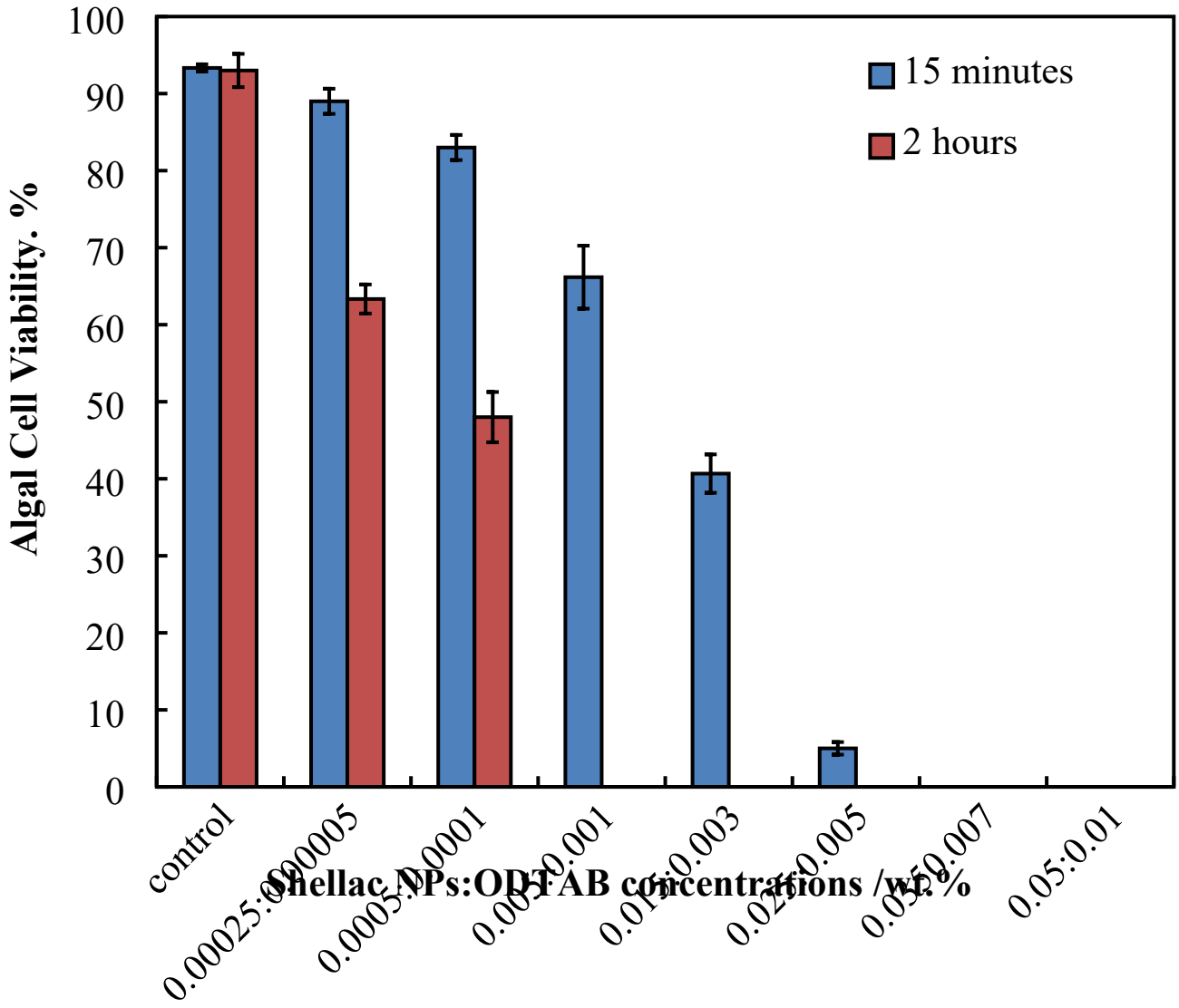


Figure S8: The viability of *C. reinhardtii* cells upon incubation for 15 min, and 2 hours at pH 5.5 with different amounts of Shellac NPs coated with ODTAB at room temperature, (n=3).

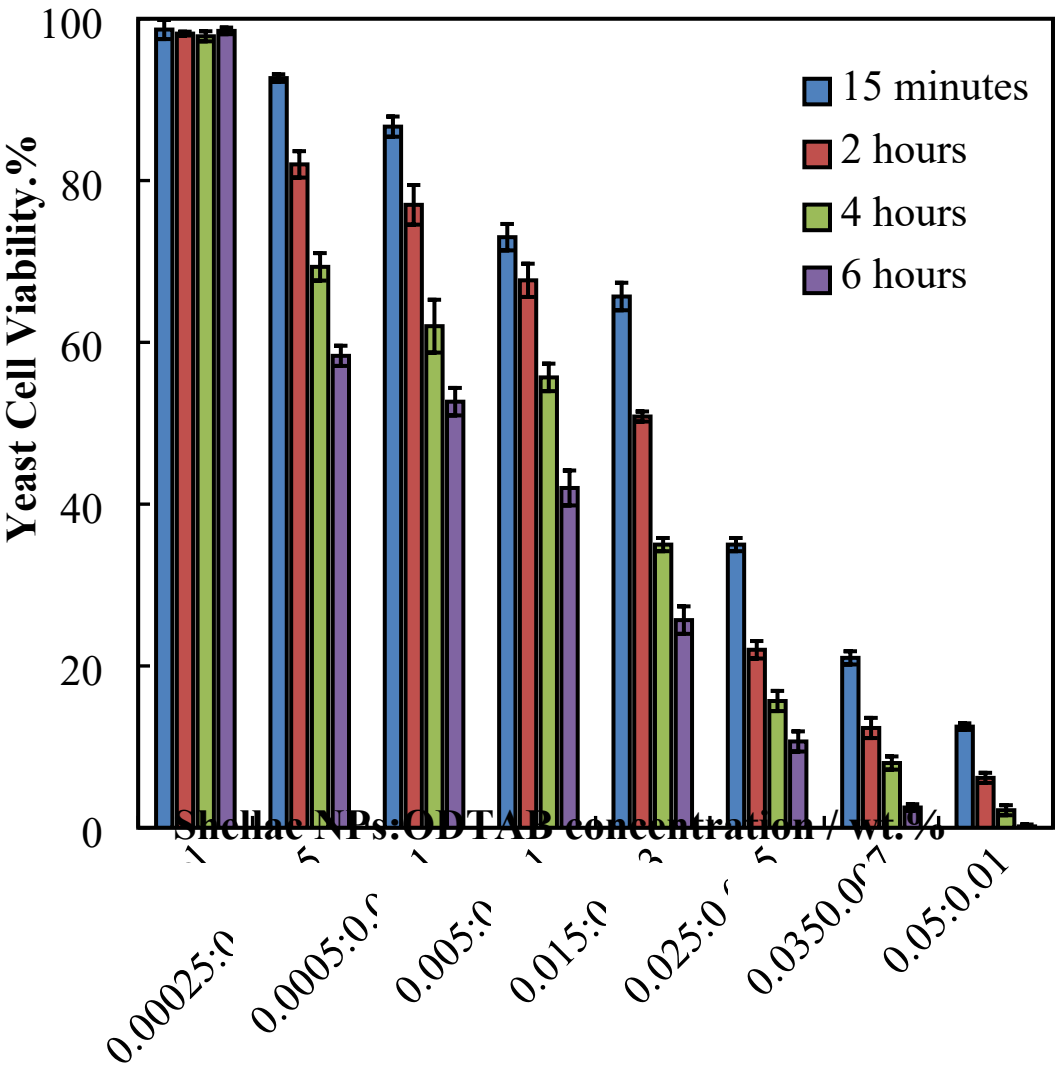


Figure S9: The viability of yeast cells incubated with non-loaded ODTAB-coated shellac NPs of different concentrations at different incubation times: for 15 min, 2 h, 4 h, and 6 h at pH 5.5. Viability is assessed using the FDA assay, (n=3).

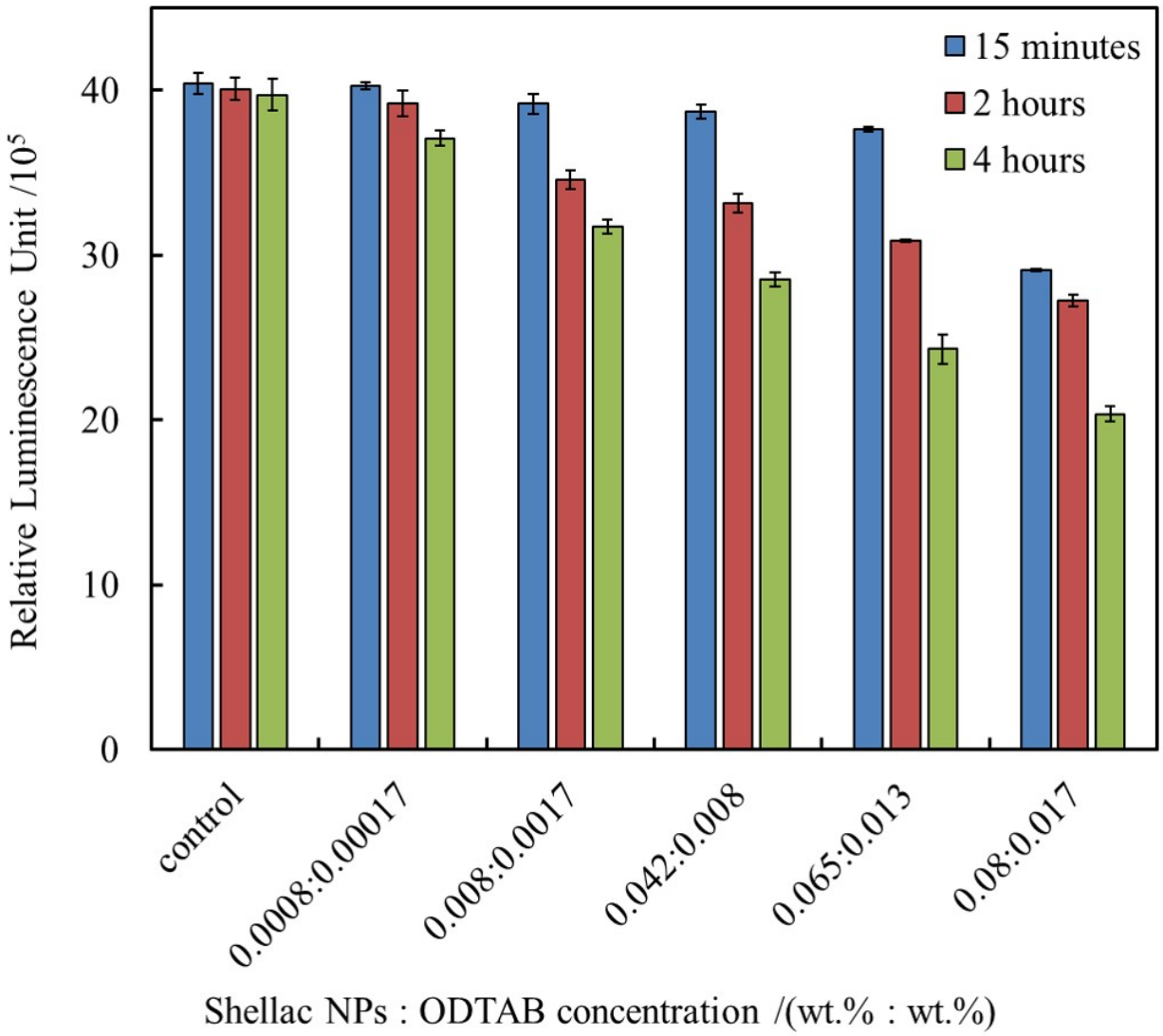


Figure S10. The cytotoxic effect of non-loaded ODTAB-coated shellac NPs of different concentrations on *E. coli* for several different incubation times at room temperature. The shellac NPs were not loaded with CUR. The ratio of shellac:ODTAB in the NPs is fixed to 5:1. The *x*-axis shows the variation of the shellac and ODTAB concentrations for these experiments.

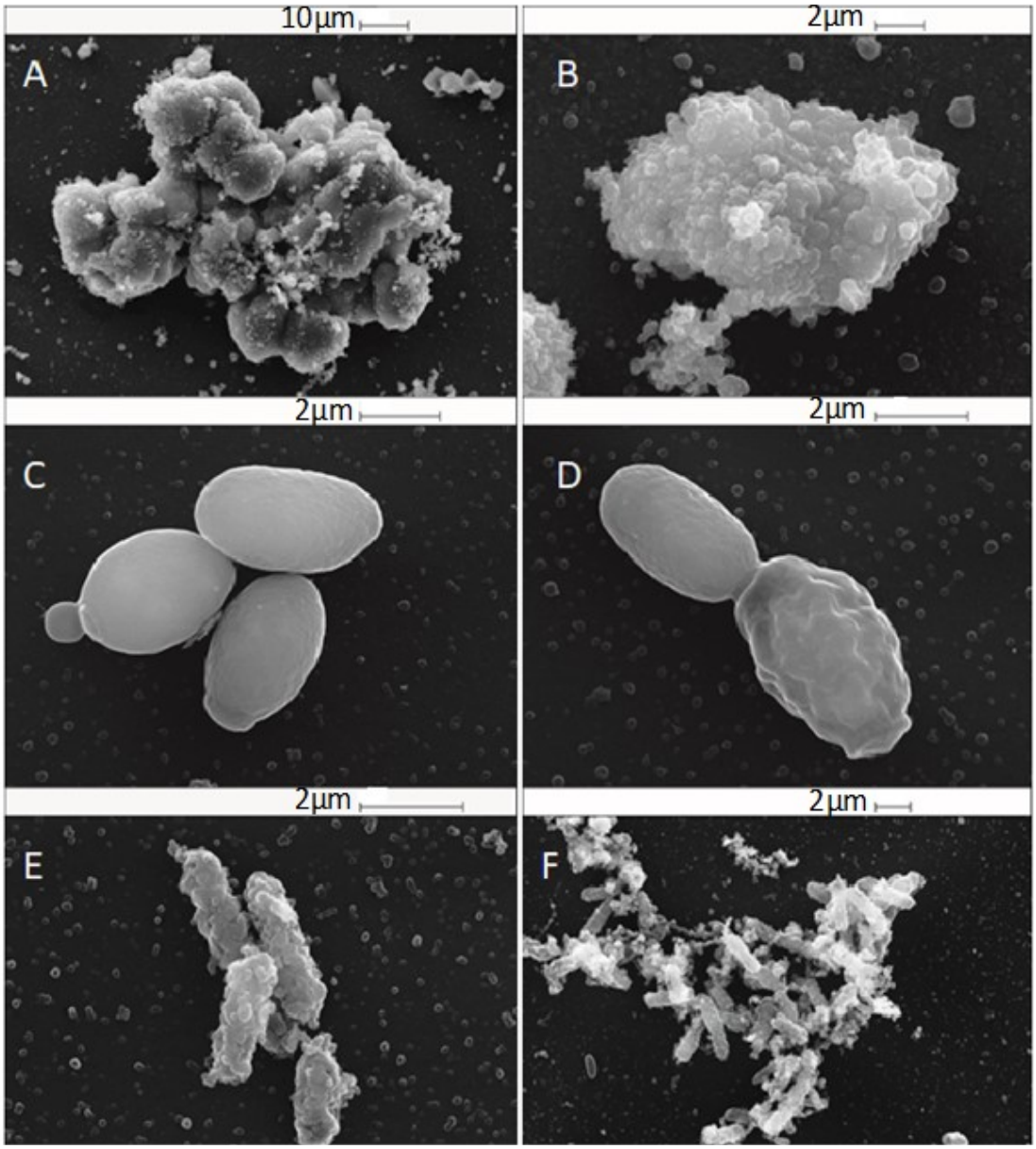


Figure S11: SEM images of microbial cells after being incubated with 0.025 wt.% ODTAB coated 0.125 wt.% shellac NPs for 4 hours at pH 5.5. (A,B) *C. reinhardtii* algal cells, (C,D) yeast cells, and (E,F) *E.coli* cells.

Table S1: Statistical analysis data for the effect of ODTAB-coated CUR NPs on microalgae based on the results in Figure 4E.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	
Control vs. 0.0005 wt.% CUR-NPs	15	6.967 to 23.03	Yes	**	0.0015	A-B
Control vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	28.5	20.47 to 36.53	Yes	****	<0.0001	A-C
Control vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	89.67	81.63 to 97.70	Yes	****	<0.0001	A-D
0.0005 wt.% CUR-NPs vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	13.5	5.467 to 21.53	Yes	**	0.0029	B-C
0.0005 wt.% CUR-NPs vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	74.67	66.63 to 82.70	Yes	****	<0.0001	B-D
0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	61.17	53.13 to 69.20	Yes	****	<0.0001	C-D

The control group showed significantly lower results than the 0.0005 wt.% CUR-NPs group (Mean Diff. = 15, 95% CI: 6.967 to 23.03, $p = 0.0015$), the 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB group (Mean Diff. = 28.5, 95% CI: 20.47 to 36.53, $p < 0.0001$), and the 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB group (Mean Diff. = 89.67, 95% CI: 81.63 to 97.70, $p < 0.0001$). Similarly, the 0.0005 wt.% CUR-NPs group was significantly different from both the 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB group (Mean Diff. = 13.5, 95% CI: 5.467 to 21.53, $p = 0.0029$) and the 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB group (Mean Diff. = 74.67, 95% CI: 66.63 to 82.70, $p < 0.0001$). Finally, significant differences were observed between the 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB and 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB groups (Mean Diff. = 61.17, 95% CI: 53.13 to 69.20, $p < 0.0001$).

Table S2: Statistical analysis data for the effect of ODTAB-coated CUR NPs on yeast based on the results in Figure 5E.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control					
15 min vs. 2 h	0.5000	-2.824 to 3.824	No	ns	0.7539
0.0005 wt.% CUR-NPs					
15 min vs. 2 h	1.333	-1.990 to 4.657	No	ns	0.4077
0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB					
15 min vs. 2 h	5.333	2.010 to 8.657	Yes	**	0.0036
0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB					
15 min vs. 2 h	10.00	6.676 to 13.32	Yes	****	<0.0001
15 min					
Control vs. 0.0005 wt.% CUR-NPs	7.667	3.181 to 12.15	Yes	***	0.0008
Control vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	25.67	21.18 to 30.15	Yes	****	<0.0001
Control vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	86.33	81.85 to 90.82	Yes	****	<0.0001
0.0005 wt.% CUR-NPs vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	18.00	13.51 to 22.49	Yes	****	<0.0001
0.0005 wt.% CUR-NPs vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	78.67	74.18 to 83.15	Yes	****	<0.0001
0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	60.67	56.18 to 65.15	Yes	****	<0.0001
2 h					
Control vs. 0.0005 wt.% CUR-NPs	8.500	4.014 to 12.99	Yes	***	0.0003
Control vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	30.50	26.01 to 34.99	Yes	****	<0.0001
Control vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	95.83	91.35 to 100.3	Yes	****	<0.0001
0.0005 wt.% CUR-NPs vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	22.00	17.51 to 26.49	Yes	****	<0.0001
0.0005 wt.% CUR-NPs vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	87.33	82.85 to 91.82	Yes	****	<0.0001
0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	65.33	60.85 to 69.82	Yes	****	<0.0001

The analysis revealed no significant temporal changes within the Control group or the group treated with 0.0005 wt.% CUR-NPs between the 15-minute and 2-hour time points. In contrast, pronounced

time-dependent differences were observed in the groups treated with 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB and 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB. Among these, the 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB group exhibited the most substantial changes across all time points compared to both the Control and the uncoated 0.0005 wt.% CUR-NPs group. Furthermore, at both 15 minutes and 2 hours, the Control group showed significant differences when compared with all other treatment groups, again with the 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB displaying the largest mean differences. Notably, consistent and significant differences were also found between the 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB and the 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB groups, indicating a clear and persistent distinction in their effects at both assessed time points.

Table S3: Statistical analysis data for the effect of ODTAB-coated CUR NPs on E.coli based on the results in Figure 6E.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control					
15 min vs. 2 h	1.757	0.02231 to 3.491	Yes	*	0.0467
15 min vs. 4 h	1.747	0.01231 to 3.481	Yes	*	0.0482
2 h vs. 4 h	-0.01000	-1.744 to 1.724	No	ns	0.9999
0.0005 wt.% CUR-NPs					
15 min vs. 2 h	5.407	3.672 to 7.141	Yes	****	<0.0001
15 min vs. 4 h	11.63	9.892 to 13.36	Yes	****	<0.0001
2 h vs. 4 h	6.220	4.486 to 7.954	Yes	****	<0.0001
0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB					
15 min vs. 2 h	5.527	3.792 to 7.261	Yes	****	<0.0001
15 min vs. 4 h	10.16	8.426 to 11.89	Yes	****	<0.0001
2 h vs. 4 h	4.633	2.899 to 6.368	Yes	****	<0.0001
Row 4					
15 min vs. 2 h	13.42	11.69 to 15.15	Yes	****	<0.0001
15 min vs. 4 h	22.05	20.32 to 23.79	Yes	****	<0.0001
2 h vs. 4 h	8.634	6.900 to 10.37	Yes	****	<0.0001
15 min					
Control vs. 0.0005 wt.% CUR-NPs	2.757	0.8408 to 4.673	Yes	**	0.0030
Control vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	4.960	3.044 to 6.876	Yes	****	<0.0001
Control vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	10.55	8.632 to 12.46	Yes	****	<0.0001
0.0005 wt.% CUR-NPs vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	2.203	0.2875 to 4.119	Yes	*	0.0200
0.0005 wt.% CUR-NPs vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	7.791	5.875 to 9.707	Yes	****	<0.0001
0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB vs. Row 4	5.588	3.672 to 7.503	Yes	****	<0.0001
2 h					
Control vs. 0.0005 wt.% CUR-NPs	6.407	4.491 to 8.323	Yes	****	<0.0001
Control vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	8.730	6.814 to 10.65	Yes	****	<0.0001
Control vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	22.21	20.30 to 24.13	Yes	****	<0.0001
0.0005 wt.% CUR-NPs vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	2.323	0.4075 to 4.239	Yes	*	0.0134
0.0005 wt.% CUR-NPs vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	15.80	13.89 to 17.72	Yes	****	<0.0001
0.0042 wt.% shellac NPs coated	13.48	11.57 to 15.40	Yes	****	<0.0001

with 0.0008 wt.% ODTAB vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB					
4 h					
Control vs. 0.0005 wt.% CUR -NPs	12.64	10.72 to 14.55	Yes	****	<0.0001
Control vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	13.37	11.46 to 15.29	Yes	****	<0.0001
Control vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	30.86	28.94 to 32.77	Yes	****	<0.0001
0.0005 wt.% CUR-NPs vs. 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB	0.7367	-1.179 to 2.653	No	ns	0.7160
0.0005 wt.% CUR-NPs vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	18.22	16.30 to 20.13	Yes	****	<0.0001
0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB vs. 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB	17.48	15.57 to 19.40	Yes	****	<0.0001

The analysis revealed significant differences within each group at all time points, with 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB showing the highest mean differences relative to the Control and other groups at all time points. Most pairwise comparisons between groups at each time point were significant, with the 0.0005 wt.% CUR-NPs and 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB groups consistently differing from the Control and 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB groups. The 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB group showed intermediate results, with some significant differences compared to other groups but less pronounced than 0.0005 wt.% CUR-NPs coated with 0.0008 wt.% ODTAB. No significant difference was observed between 0.0005 wt.% CUR-NPs and 0.0042 wt.% shellac NPs coated with 0.0008 wt.% ODTAB at 4 hours.