Electronic supplementary information

Variation in surface properties, metabolic capping, and antibacterial activity of biosynthesized silver nanoparticles: comparison of bio-fabrication potential in phytohormone-regulated cell cultures and naturally grown plant.

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1. Materials and Methods:

1.1. Protocol for Metabolite profiling of AgNPs using LC-MS/MS

All the samples were analyzed on an Agilent 6220A Time-of-Flight LC/MS operated with (+) ESI-MS for the profiling of secondary metabolites associated with the AgNPs. The Mass spectroscopy system was coupled with the HPLC system Agilent 1100/1200 series system consisting of G1367C HiP-ALS SL autosampler, G1330B FC/ALS Therm thermostat cooler for autosampler, G1379B degasser, and G1312B binary pump.

A ThermoScientific acclaim polar advantage column (C16, 3µm, 2.1 x 150 mm 120 A + guard). was used for LC separation of metabolites for LC-MS analysis. For LC, the mobile phase contained A = H_2O + 0.1% formic acid and B = 100% Methanol + 0.1% formic acid. The samples were loaded onto the column using 100% A (0 −20 min) at a flow rate of 200 µL/min and resolved using a linear increasing gradient of 100% B to 0% A for 24 min (20 −24 min). The gradient for Solvent B was decreased to 0 % B for 35 min (25-35 min).

For MS analysis the positive- (+) ion data were generated by ESI in the mass spectrophotometer. The following conditions were maintained during the process: capillary voltage (3000 V), charging (2000 V), corona (2 µA), drying gas flow (8 l/min), fragmentor (120 V), gas temperature (300°C), nebulizer (45 PSIG), octopole radio frequency (250 V), skimmer (60 V), and vaporizer temperature (200°C). The mass spectra were acquired in 4 GHz high-resolution mode at a rate of 1.02 spectra/s and 9,700 transients/spectrum, and data were collected as profiled spectra untargeted metabolites. The data obtained were further analyzed through search in the METLIN database through XCMS online Version 3.7.1 developed by the Scripps institute (https://xcmsonline.scripps.edu/). The datasets (WPE-AgNPs; 3 reps, TDZ-AgNPs; 3 reps and MLN-AgNPs; 3 reps) were uploaded to the XCMS online server. The data were analyzed by comparing all treatments in a two-way combination i.e. WPE-AgNPs vs

TDZ, TDZ-AgNPs vs MLN-AgNPs; and WPE-AgNPs Vs MLN-AgNPs) for the common and unique metabolites obtained from the samples. The putative identifications are based on a METLIN search of the accurate mass selected with tolerance in mass difference ≤10 ppm.

Table S1: Putative metabolites detected in extracts from TDZ-, MLN- and WPE-AgNPs through LC-MS/MS-based metabolite profiling

Feature id	Putative molecules	Corresponding class of metabolites	Label	Adducts	METLIN ID	m/z	Mass difference tolerance (ppm)	Retention time (seconds)	Feature intensity					
									TDZ-AgNPs mean	TDZ-AgNPs (sd)	MLN-AgNPs mean	MLN-AgNPs (sd)	WPE-AgNPs mean	WPE-AgNPs (sd)
1.	11-Aminoundecanoic acid	Fatty acids	*	M+H	265158	202.181	5	34.707	5549139.340	9609563.844	0	0	0	0
2.	Clostebol propionate	Fatty acid	*	M+H	453424	379.203	0	4.481	33104.789	34985.108	0	0	0	0
3.	Lauryldiethanolamine	Amines	$	M+H	305368	274.273	4	22.317	0	0	209826.572	8772.691	0	0
4.	Somocystinamide A	Lipopeptide	$	M+H	65448	627.398	1	27.102	0	0	434539.834	24603.487	0	0
5.	Asp-Lys-Trp-Trp	Polypeptide	$	M+K	123058	672.254	0	31.247	0	0	1980.376	190.846	0	0
6.	*N,N-dimethyl-1,1-bis(2-trimethylsilylethoxy)methanamine*	Amines	$	M+H	489395	292.213	1	31.626	0	0	2780.647	456.141	0	0
7.	5-Benzyl 1-methyl N-hexadecanoyl-L-glutamate	Amino acid derivative	$	M+Na	950451	512.334	1	26.413	0	0	8249.517	1577.460	0	0
8.	2-Decarboxyphyllocactin	Glycosides (Betalains)	$	M+H	91522	594.169	0	27.296	0	0	75585.438	16519.438	0	0
9.	Correolide	Nortriterpenoid	$	M+Na	69731	811.317	3	31.501	0	0	1003.384	263.284	0	0
10.	Litcubinine	Alkaloid	$	M+Na	88591	337.128	0	23.718	0	0	2295.927	631.041	0	0
11.	*N,N-Bis[4-(naphthalen-1-yl)phenyl]perylen-3-amine*	Amines	$	M+Na	975862	694.248	3	31.237	0	0	1645.659	460.575	0	0
12.	*Trimethylolpropane diacrylate 3-(hexamethyleniminyl)propionate*	Fatty acids	$	M+NH4	378961	413.265	0	26.715	0	0	365045.694	120528.412	0	0
13.	*1-[4-(3-phenyl-3H-naphtho[2,1-b] pyran-3-yl)phenyl] Pyrrolidine*	Amines	$	M+Na	634547	426.183	0	26.040	0	0	23763.591	8397.453	0	0
14.	1-octadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine	Amines	$	M+K	40425	784.525	1	27.877	0	0	2434080.937	908716.108	0	0
15.	Acrimarine N	Alkaloids	$	M+K	95256	596.167	1	27.270	0	0	30613.396	11643.463	0	0
16.	d-alpha-Tocopheryl succinate	Tocopherols	$	M+H	286740	531.406	3	27.944	0	0	1965133.353	1015534.816	0	0
17.	N,N-Dibutyltetradecan-1-amine N-oxide	Amines	$	M+H	772735	342.372	2	25.952	0	0	4699.354	2664.595	0	0

18.	N-Methyldidecylamine	Amines	$	M+H	290613	312.362	3	25.703	0	0	73927.486	42454.686	0	0
19.	N-Isodecylisotridecylamine::	Amines	$	M+H	498950	340.392	5	26.163	0	0	181842.959	108547.347	0	0
20.	Tris(tridecyl)amine	Amines	$	M+H	316253	564.641	6	26.889	0	0	71333.774	43374.634	0	0
21.	N,N-Diundecylundecan-1-amine	Amines	$	M+H	701327	480.548	6	26.113	0	0	49665.606	30199.842	0	0
22.	3-Dodecylamido-N,N'-dimethylpropyl amine oxide	Amines	$	M+H	327556	300.277	1	19.253	0	0	74649.548	48286.655	0	0
23.	3-hexanoyl-NBD Cholesterol	Sterols	$	M+H	64806	663.451	4	27.886	0	0	129531.516	83899.448	0	0
24.	Octacosanamide	Alkaloids	$	M+H	284978	424.455	8	26.388	0	0	2848.393	1856.526	0	0
25.	1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1'-rac-glycerol)	Saponin	$	M+H	40844	749.534	2	27.263	0	0	2466.305	1639.352	0	0
26.	1-(13Z,16Z-docosadienoyl)-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-glycero-3-phosphocholine Phosphatidylcholine(22:2/22:6)	Phospholipids	$	M+NH4	78450	905.595	7	26.766	0	0	66350.862	44158.651	0	0
27.	3-(Docosyloxy)-N,N-dimethylpropan-1-amine	Amines	$	M+H	911624	412.450	3	26.123	0	0	4829.722	3396.245	0	0
28.	Val Ile Val Leu Leu	Polypeptides	$	M+NH4	264339	573.433	2	27.353	0	0	3955.435	3293.090	0	0
29.	1-[(Diethoxyphosphorothioyl)oxy]-3,4,5-triiodo-1H-pyrazole	Alkaloids	$	M+K	956221	652.691	1	27.034	0	0	1581.401	1370.301	0	0
30.	N,1-Bis(tert-butoxycarbonyl)-L-histidine, compound with dicyclohexylamine (1:1)	Amino acids derivatives	$	M+NH4	471379	554.391	1	27.742	0	0	732323.002	707262.887	0	0
31.	Furohyperforin	PolyPhenols	$	M+H	89432	553.388	1	27.857	0	0	2248998.201	2211622.124	0	0
32.	1-palmitoleoyl-2-(1-enyl-vaccenoyl)-sn-glycero-3-phosphoethanolamine	Phospholipid	$	M+K	60413	738.482	3	27.844	0	0	77674.209	77739.277	0	0
33.	L-Proline, 1-(4-(((carboxymethyl)methylamino)carbonyl)-2,3,5,6-tetraiodobenzoyl)-	Amino acid derivative	ǂ	M+K	468372	876.6586463	9	26.5595	0	0	0	0	905.9404742	785.2581
34.	1-Fluoro-N,N,N',N'-tetramethyl-1-methylidene-lambda~5~-	Amines	*$	M+NH4	696914	170.121	5	16.856	285.548	270.958	1914.171	391.242	0	0

	phosphanediamine													
35.	3-Chloro-D-alanine--hydrogen chloride (1/1)	Amino acid derivative	*$	M+H	631353	159.993	1	7.081	2997.863	1405.893	8779.940	482.943	0	0
36.	2-Hexyl decanoic acid	Fatty acids	*$	M+H	24084	257.246	4	1.272	13598.226	1836.052	23831.542	3248.762	0	0
37.	D-Mannitol	Sugar alcohol	*$	M+H	277837	183.086	0	4.198	1770.436	176.556	1103.374	8.347	0	0
38.	Butanedioic acid, 2,3-dihydroxy-, bis(1-methylethyl) ester, (R,R)-(+/-)	Carboxylic acid derivative	*$	M+NH4	311504	252.144	1	24.498	763.673	1028.886	26306.183	8957.721	0	0
39.	Myristic acid ethyl ester	Fatty acids	*$	M+H	45004	257.247	3	6.217	10668.967	1779.588	27065.589	6555.240	0	0
40.	Isopalmitic acid	Polyketide	*$	M+H	4289	257.247	3	7.898	10644.196	5781.898	47980.206	20981.256	0	0
41.	4,8,12-trimethyl-tridecanoic acid	Fatty acids	*$	M+H	34669	257.246	5	8.223	20530.777	3160.877	59654.715	25702.426	0	0
42.	N,N-Dimethylpentadecan-8-amine	Amines	*$	M+H	302744	256.298	6	24.575	582.186	1008.376	38516.221	26707.264	0	0
43.	4-Ethyltetradecanoic acid	Fatty acids	*$	M+H	292794	257.246	5	0.757	53348.415	2396.406	88650.567	36401.728	0	0
44.	N,N''-1,4-butanediylbis Urea	Amide derivative	*$	M+Na	280906	197.101	1	23.674	39467.847	37881.119	2827.324	1039.853	0	0
45.	Octanoic acid, octyl ester	Fatty acids	*$	M+H	345568	257.246	4	2.004	36706.465	1868.840	67990.326	37216.392	0	0
46.	N,N'-[(Methylsilanediyl)bis(oxy)] bis(N-methylmethanamine)	Amines	*$	M+Na	885513	187.087	0	18.462	7710.679	7977.692	2556.030	888.296	0	0
47.	7-Methoxychromone	Phenylpropanoids	*$	M+H	44466	177.055	2	5.692	33211.033	51108.549	2797.158	627.428	0	0
48.	Ile Met Val Tyr	Polypeptide	*‡	M+Na	163838	547.2559349	0	24.666	12526.44178	21696.43361	0	0	42704.80148	8536.443
49.	Arg Trp Phe	Polypeptides	*‡	M+H	20924	508.2666883	0	24.47383	16669.88997	28873.09639	0	0	52958.41577	15702.62
50.	beta-D-Galactopyranoside	Monosaccharides	*‡	M+K	577112	799.4391743	0	23.81633	4153.76522	7194.532404	0	0	13391.02221	1406.326
51.	Galactosyl Ceramide	Glycolipids	*‡	M+K	64958	896.6947905	0	28.26267	47194.11607	81742.60686	0	0	706192.503	517865.2
52.	L-Lysyl-L-lysyl-L-tyrosyl-L-leucine	Polypeptides	*‡	M+H	907192	551.3543041	2	24.483	1342.233109	2324.81594	0	0	27467.65757	21607.59
53.	Lauryldiethanolamine::M+H::305368::D::|1::Octanoic acid, compd. with 1-octanamine (1:1)	Amines	*‡	M+H	359700	274.2737878	1	21.369	8280.484741	14342.22028	0	0	149413.0367	118493.8
54.	4-nitro-1,2,3-trimethyl-1H-Imidazolium	Alkaloids	*‡	:M+H	319769	157.0842698	2	28.99467	27064.14618	30001.81358	0	0	72716.09205	367300.98
55.	2,5-Difluoro-1H-imidazole-4-carboxylic acid	Alkaloids	*‡	M+H	371142	149.0157958	1	30.83925	18891.28208	20675.34807	0	0	17391.51654	31033.954

56.	1,3-Bis(propoxymethyl)-2,3-dihydro-1H-imidazol-1-ium chloride	Alkaloids	*ǂ	M+H	893229	250.1442646	0	17.28025	37128.81249	64308.98965	0	0	65346.91776	18461.74
57.	punaglandin 5	Fatty acids	*ǂ	M+K	36424	537.1652509	0	26.3765	2255.184472	3906.094087	0	0	129431.7816	107661
58.	5-Diethylamino-2-pentanone	Ketone derivative	*ǂ	M+H	270259	158.1544564	3	21.028	155.9843663	135.0945814	0	0	3378.379894	3086.605
59.	2-(Diphenylmethyl)-5-phenyl-1H-benzo[e]isoindole-1,3(2H)-dione	Alkaloids	*ǂ	M+Na	653347	462.1462435	0	25.84633	1360.717446	2356.83175	0	0	94479.06557	91460.54
60.	3,6,9,12,15-Pentaoxaheptadecan-1-ol, 17-(nonylphenoxy)-	Fatty acids	*ǂ	M+NH4	363692	502.3738006	0	24.4995	848.4587421	1469.573649	0	0	8087.895137	7579.478
61.	1,2,3,4,5,6,7,8-octahydro-Xanthylium.	Polyphenols	*ǂ	M+Na	503715	212.116755	2	16.14017	1399.394013	2282.996149	0	0	4383.598766	3504.997
62.	N-Methyl-N-octadecyl-1-octadecanamine	Fatty acids	*ǂ	M+H	290222	536.6109955	4	25.24217	19424.01303	33643.37745	0	0	134907.9419	149353.8
63.	L-Tryptophyl-L-lysyl-L-tyrosyl-L-methionyl-L-tryptophyl-L-valine	Polypeptides	*ǂ	M+Na	970332	934.430677	6	30.63458	270.7883713	469.0192173	0	0	837.8855266	732.0389
64.	N-(2-aminoethyl)-N-[2-[(1-oxooctadecyl)amino]ethyl]-Octadecanamide	Fatty acids	*ǂ	M+Na	374729	658.6217831	0	24.88967	2251.577767	3899.84709	0	0	12853.05142	14888.35
65.	2-[benzoyl(2-dodecanoyloxyethyl)amino] ethyl dodecanoate	Fatty acids	*ǂ	M+K	564285	612.4021162	1	24.575	1484.53359	2571.287603	0	0	3337.320516	1308.381
66.	1-[(2-Methoxyphenyl)methyl]piperidine-4-carboxylic acid	Alkaloids	*ǂ	M+H	267180	250.1433499	2	16.51083	31186.61963	53832.43095	0	0	57877.26484	8487.15
67.	N~1~-(3-Aminopropyl)butane-1,4-diamine--hydrogen chloride (1/1)	Amines	*ǂ	M+NH4	739863	199.1684025	0	1042	2130.588942	325.1582551	0	0	2445.698703	626.6337
68.	Ethyl 2-(diethylcarbamoyl) benzoate	Alcohol ester	*ǂ	M+H	271154	250.1439207	1	16.9485	27743.85723	47868.83679	0	0	49874.84597	9769.037
69.	1-Tetradecanamine, N-methyl-N-tetradecyl	Amines	*ǂ	M+H	366932	424.4852655	6	24.28283	3516.774083	6091.231391	0	0	11030.22277	16464.45
70.	Bisazir	Amines	*ǂ	M+H	330537	178.0562348	0	29.35567	2290.220895	1320.951923	0	0	1734.673907	614.2076
71.	11Z-Hentriacontene	Alkane hydrocarbon	$ǂ	M+NH4	97485	452.515	8	25.896	0	0	25548.186	17781.674	16957.97715	21172.59
72.	Loroxanthin ester/ Loroxanthin dodecenoate	Terpenes	$ǂ	M+K	41412	803.540	3	26.715	0	0	82304.782	48559.587	44927.03505	40582.62
73.	Lantadene B	Terpenes	$ǂ	M+H	506610	553.388	1	28.122	0	0	1825542.087	970784.074	1246215.694	1422537

74.	N,N-Dimethyl-1-octadecanamine	Amines	$ǂ	M+H	288395	298.346	2	25.407	0	0	263332.136	135688.135	147443.6187	116084.4
75.	2-[(E)-2-(Acridin-9-yl)ethenyl]-5-(diethylamino)phenyl octadecanoate	Fatty acids	$ǂ	M+NH4	739598	652.480	6	27.944	0	0	4559270.804	2233671.821	2486784.684	2045603
76.	N-heptadecyl-1-Octadecanamine	Amines	$ǂ	M+H	589281	524.575	4	26.446	0	0	16323.450	8056.867	12137.06687	11237.65
77.	2-Tritriacontanone	Ketone	$ǂ	M+NH4	355694	496.547	4	26.263	0	0	2380.893	1126.865	3523.468965	4251.985
78.	2-(Pentacosylcarbamoyl)benzoic acid	Aromatic carboxylic acids	$ǂ	M+K	951729	554.400	6	25.967	0	0	77394.467	32820.011	57233.48575	45894.7
79.	Arginine	Amino Acid	*$ǂ	M+Na	274389	197.101	0	22.850	30312.935	29167.851	378.595	167.063	933.0944293	238.2348
80.	14-Amino-3,6,9,12-tetraoxatetradecan-1-oic acid	Fatty acid	*$ǂ	M+H	294075	252.144	0	24.988	1319.085	1147.958	46992.132	11256.487	33676.71269	2411.425

Labels: *Compounds detected only in TDZ-AgNPs (1-2); $MLN-AgNPs only (3-32); ǂWPE-AgNPs only (33); *$both in TDZ and MLN-AgNPs (34-47); *ǂTDZ- and WPE-AgNPs (48-70); $ǂMLN and WPE-AgNPs (71-78). *$ǂDetected in All (79-80).

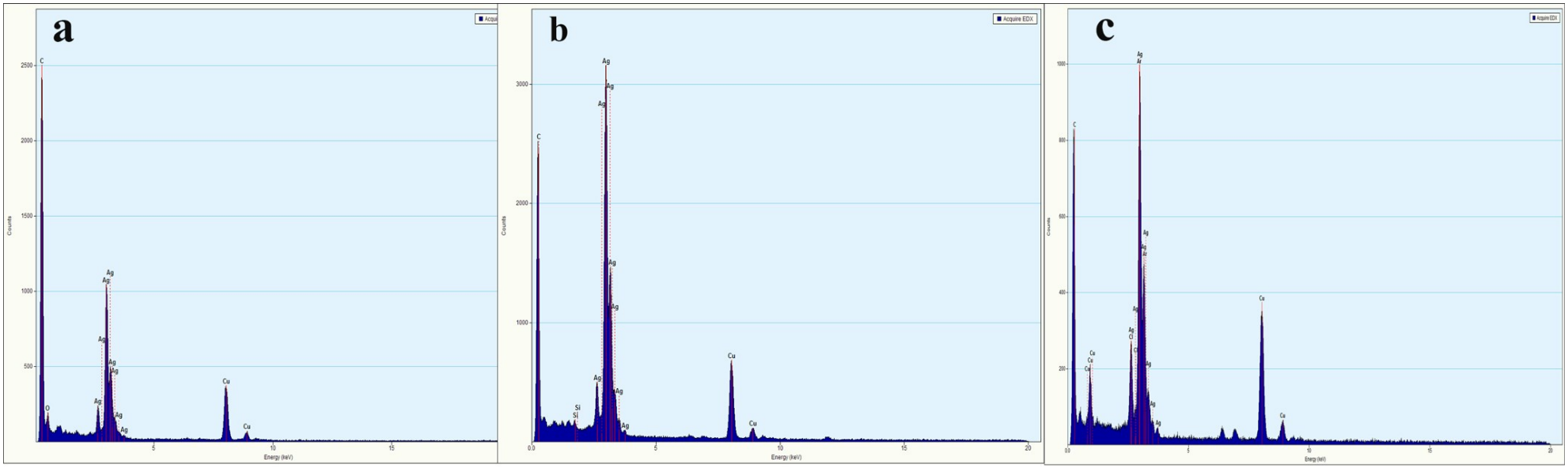

Figure S1: EDX spectra of (a) TDZ-, (b) WPE- and (c) MLN-AgNPs, respectively showing the characteristic peak for Ag. Peaks of C, Si, and Cu, which might result from secondary metabolites in biological extracts are also shown.

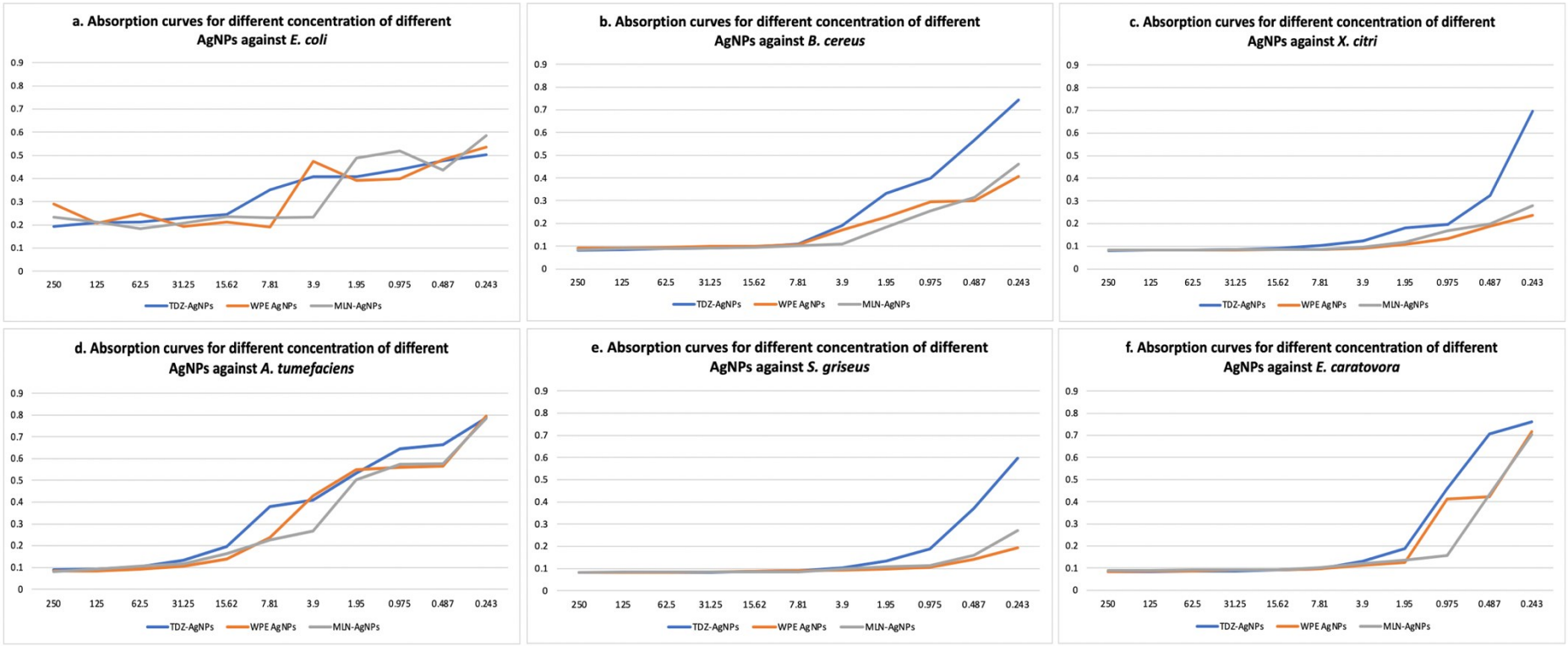

Figure S2: Differential response in terms of absorption of light at OD625 nm during antibacterial activity of MLN-, TDZ- and WPE-AgNPs

Table S2: Data on absorption response at OD625 nm during antibacterial activity of MLN-, TDZ- and WPE-AgNPs

AgNPs Concentration (ug/mL)												
Types of AgNPs	***E. coli***											
	250	**125**	**62.5**	**31.25**	**15.62**	**7.81**	**3.9**	**1.95**	**0.975**	**0.487**	**0.243**	
TDZ-AgNPs	0.193	0.21	0.212	0.231	0.245	0.351	0.408	0.408	0.439	0.476	0.503	
WPE AgNPs	0.29	0.207	0.247	0.194	0.212	0.191	0.475	0.391	0.398	0.481	0.536	
MLN-AgNPs	0.234	0.213	0.183	0.207	0.236	0.232	0.233	0.489	0.52	0.436	0.586	
B. cereus												
TDZ-AgNPs	0.083	0.085	0.09	0.091	0.096	0.11	0.192	0.332	0.399	0.568	0.743	
WPE AgNPs	0.092	0.092	0.094	0.1	0.1	0.106	0.17	0.228	0.294	0.3	0.407	
MLN-AgNPs	0.085	0.089	0.089	0.093	0.094	0.101	0.11	0.183	0.256	0.315	0.461	
X. citri												
TDZ-AgNPs	0.081	0.083	0.083	0.086	0.09	0.104	0.123	0.181	0.197	0.324	0.695	
WPE AgNPs	0.083	0.083	0.083	0.084	0.085	0.085	0.09	0.109	0.134	0.188	0.237	
MLN-AgNPs	0.083	0.084	0.084	0.085	0.086	0.086	0.095	0.119	0.17	0.2	0.28	
A. tumefaciens												
TDZ-AgNPs	0.089	0.092	0.104	0.133	0.197	0.379	0.41	0.533	0.646	0.663	0.787	

WPE AgNPs	0.085	0.085	0.091	0.105	0.139	0.236	0.429	0.548	0.56	0.566	0.795	
MLN-AgNPs	0.082	0.091	0.105	0.118	0.162	0.227	0.268	0.502	0.574	0.576	0.786	
S. griseus												
TDZ-AgNPs	0.082	0.083	0.083	0.083	0.088	0.091	0.102	0.135	0.189	0.371	0.596	
WPE AgNPs	0.083	0.083	0.083	0.086	0.088	0.091	0.092	0.099	0.106	0.143	0.194	
MLN-AgNPs	0.082	0.084	0.085	0.086	0.086	0.086	0.099	0.108	0.114	0.16	0.271	
E. caratovora												
TDZ-AgNPs	0.084	0.085	0.086	0.086	0.091	0.098	0.13	0.188	0.459	0.707	0.762	
WPE AgNPs	0.083	0.086	0.086	0.091	0.091	0.096	0.112	0.126	0.411	0.423	0.718	
MLN-AgNPs	0.09	0.09	0.091	0.092	0.092	0.102	0.119	0.136	0.156	0.433	0.703	