Supplementary Information

Phytochemical-based nanocomposites for the treatment of bacterial biofilms

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Size distribution of nanocomposites

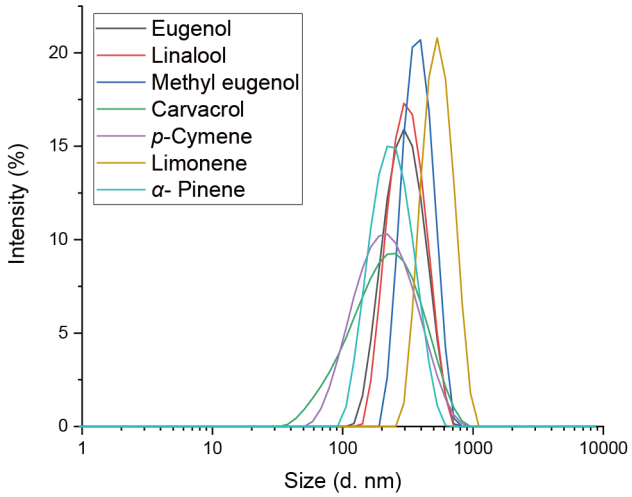

Figure S1 Average diameters of nanocomposites loaded with different phytochemicals in phosphate buffer saline (150 mM) were determined by DLS (Malvern Zetasizer Nano ZS)

Antimicrobial activity of nanocomposites and phytochemicals against bacterial biofilms

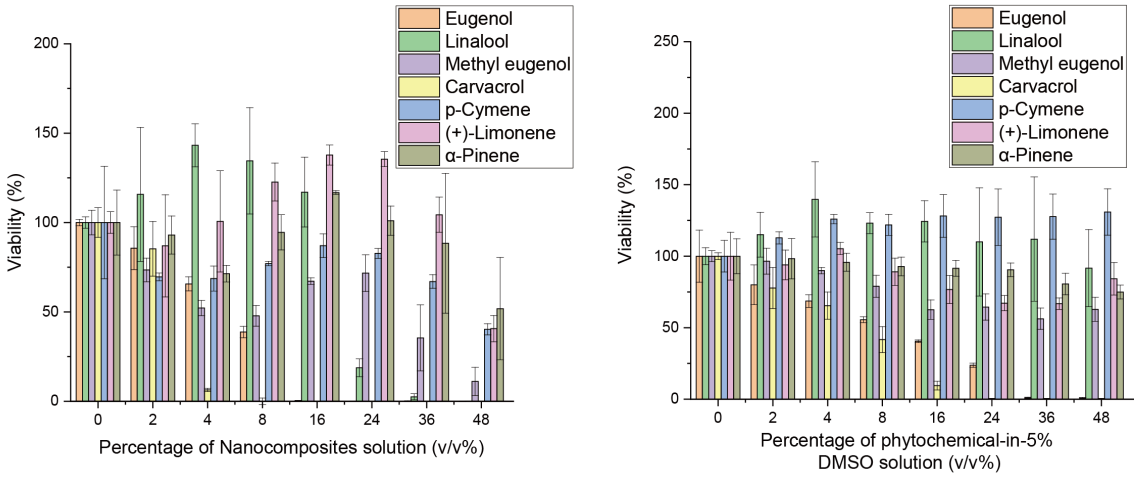

Figure S2 Viabilities of ***E. coli*** **(CD2)** biofilms after a three-hour treatment with a) NCs or b) phytochemicals in 5 v/v% DMSO solution. Data were presented as mean ± standard deviation and represented three independent experiments.

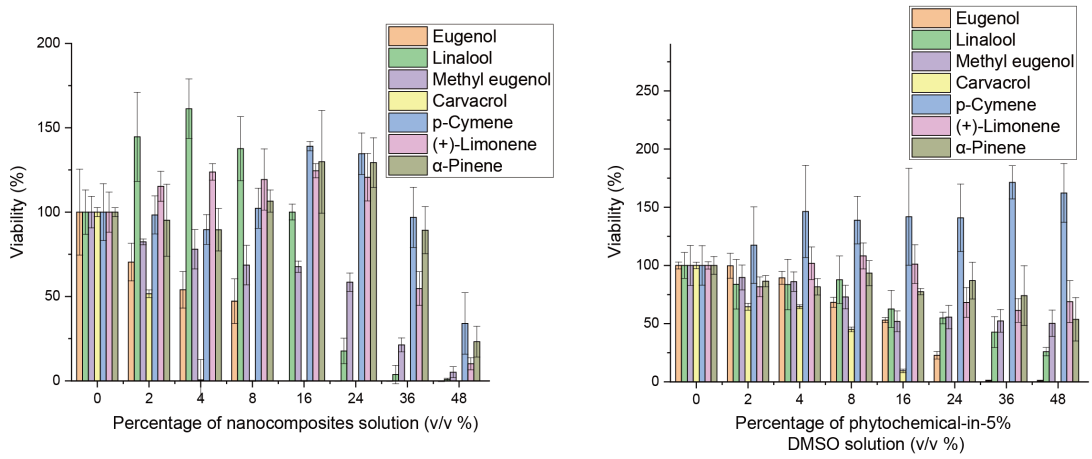

Figure S3 Viabilities of ***P. aeruginosa*** **(CD1006)** biofilms after a three-hour treatment with a) NCs or b) phytochemicals in 5 v/v% DMSO solution. Data were presented as mean ± standard deviation and represented three independent experiments.

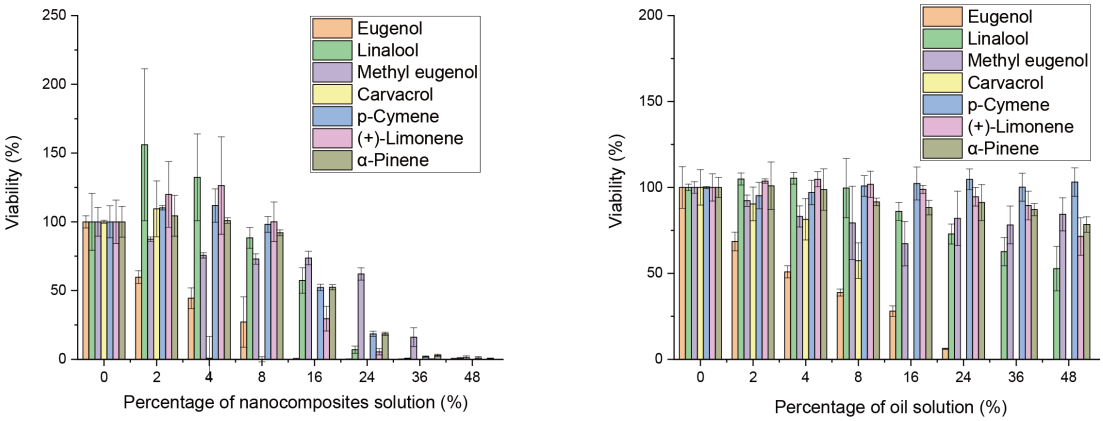

Figure S4 Viabilities of ***E. cloacae*** **complex (CD1412)** biofilms after a three-hour treatment with a) NCs or b) phytochemicals in 5 v/v% DMSO solution. Data were presented as mean ± standard deviation and represented three independent experiments.

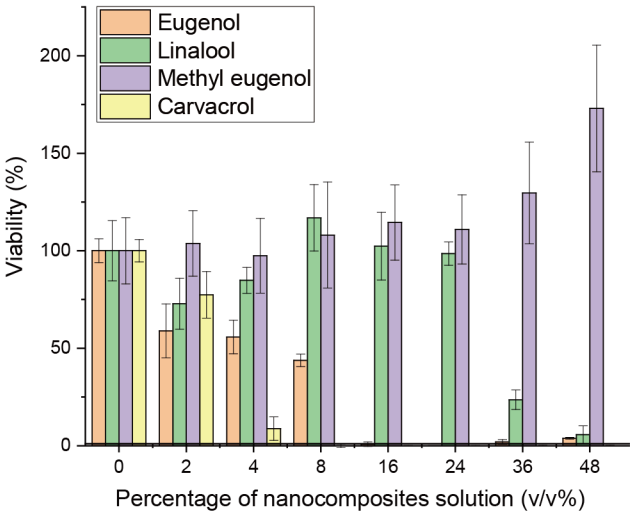

Figure S5 Viabilities of ***S. aureus*** **(CD489, MRSA)** biofilms after a three-hour treatment with nanocomposites loaded with eugenol, linalool, methyl eugenol, or carvacrol. Data were presented as mean ± standard deviation and represented three independent experiments.

Biomass of bacterial biofilms after NCs treatment

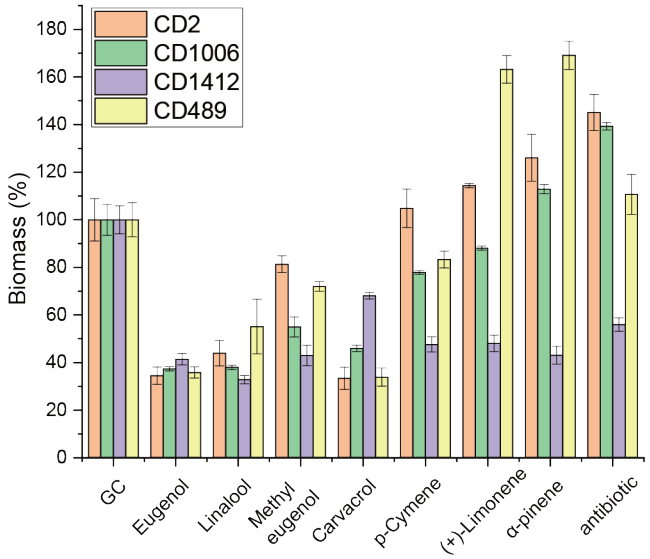

Figure S6 Biomass of ***E. coli*** **(CD2),** ***P. aeruginosa*** **(CD1006)**, ***E. cloacae*** **complex (CD1412),** and ***S. aureus*** (**CD489**) biofilms after a three-hour treatment with NCs. The concentrations were either the corresponding $MBEC_{90}$ of the NCs (if applicable) or 48 v/v%. Furthermore, biofilms were treated with antibiotics as controls. Specifically, Gram-negative bacterial biofilms were treated with 10 × MIC of colistin while Gram-positive biofilms were treated with 10 × MIC of vancomycin. Data were presented as mean ± standard deviation and represented three independent experiments.

Cytotoxicity of nanocomposites to 3T3 fibroblast cells

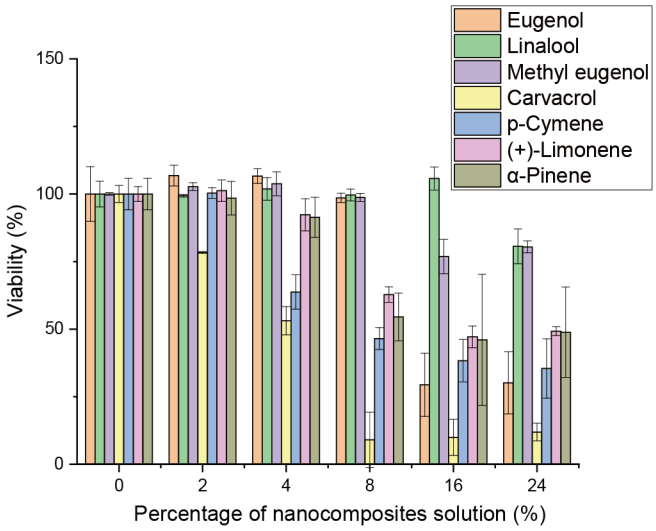

Figure S7 Viabilities of **3T3 fibroblast** cells after a three-hour treatment with NCs. The viabilities were determined using Pierce LDH cytotoxicity assay. Data were presented as mean ± standard deviation and represented three independent experiments.

$MBEC_{90}$ and GI_{50} of Nanocomposites

Encapsulated phytochemical	$MBEC_{90}$ (v/v %)				GI_{50} (v/v %)
	CD2 *E. coli*	CD1006 *P. aeruginosa*	CD1412 *E. cloacae* complex	CD489 *S. aureus*	3T3 Fibroblast cell
Eugenol	13.8	11.86	12.23	9.27	8.89
Linalool	25.98	26.83	30.03	37.9	27.14
Methyl eugenol	nd	43.21	39.14	nd	nd
Carvacrol	3.44	2.96	2.22	3.55	3.9
***p*-Cymene**	nd	nd	28.88	nd	6.62
Limonene	nd	nd	21.64	nd	13.88
α-pinene	nd	nd	29.22	nd	9.83

Table S1 NCs' minimum concentration to eradicate 90% of biofilms ($MBEC_{90}$) against four bacteria strains and their concentrations to inhibit 50% fibroblast cell proliferation (GI_{50}). The abbreviation "nd" indicated not determined.

Bacterial strain information

	Riley Strain Name	CD-2	CD-1412	CD-1006	CD-489
	Species	*E. coli*	*E. cloacae complex*	*P. aeruginosa*	*S. aureus - MRSA*
	Date Isolated	9/11/2011	7/12/2006	4/23/2012	3/12/2001
	Specimen	UCC	UCC	UCC	UCS
	CFU/mL	>100,000	>100,000	>100,000	>100,000
	Note		Urine from nephrostomy tube		
Aminoglycosides	**Amikacin** (Amikin)		S		
	Gentamicin (Garamycin)	S	I	S	S
	Kanamycin High Level				
	Tobramycin (Nebcin)		R		
β-Lactam	**Ampicillin** (Omnipen, Polycillin)	R		S	
	Ampicillin/sulbactam (Unasyn)	I		S	R
	Amoxicillin/CA (Augmentin)				R
	Aztreonam (Zithromax)				
	Oxacillin (Prostaphlin)				R
	Penicillin				R
	Piperacillin (Pipracil)				
Cephalosporin	**Ceftazidime** (Fortaz, Tazicef)				
	Cefaclor				R

	(Ceclor, Ceclor CD)				
	Ceftriaxone (Rocephin)	S	S	S	R
	Cefotaxime				R
	Cefazolin (Ancef, Kefzol)	S	R	S	
	Ceftizoxime				
	Cefepime (Maxipime)	S	S	S	
	Cefoxitin (Mefoxin)	S	R	S	
	Cefuroxime-Sodium				R
	Cefuroxime-Axetil (Ceftin)				
Carbapenem	**Ertapenem**				
	Imipenem (Primaxin)			S	R
	Meropenem (Merrem)				
Macrolides	**Azithromycin** (Azactam)				
	Erythromycin				
Fluoroquinolone	**Ciprofloxacin** (Cipro)	S	S	S	
	Levofloxacin (Levaquin)	S	S	S	R
	Ofloxacin (Floxin)				
Lincosamides	**Clindamycin** (Cleocin)				
Oxazolidinones	**Linezolid** (Zyvox)		S		S
Antimycobacterial	**Rifampin** (Rifadin, Rimactane)				S
Folate pathway inhibitors	**Trimethoprim/Sulfa** (Gantanol)	S	R	S	S
Tetracycline	**Tetracycline**				S
Glycylcyclines	**Tigecycline**				
Glycopeptides	**Vancomycin** (Vancocin)				S

Table S2 All strains were harvested and tested for susceptibility in Cooley Dickinson Hospital Microbiology Laboratory (Northampton, MA). S: Susceptible; I: Intermediate; R: Resistant.