

SUPPLEMENTAL INFORMATION

Polyphenolic Extract from Maple Syrup Potentiates Antibiotic Susceptibility and Reduces Biofilm Formation of Pathogenic Bacteria

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Running title: Maple syrup extract exhibits antimicrobial activity

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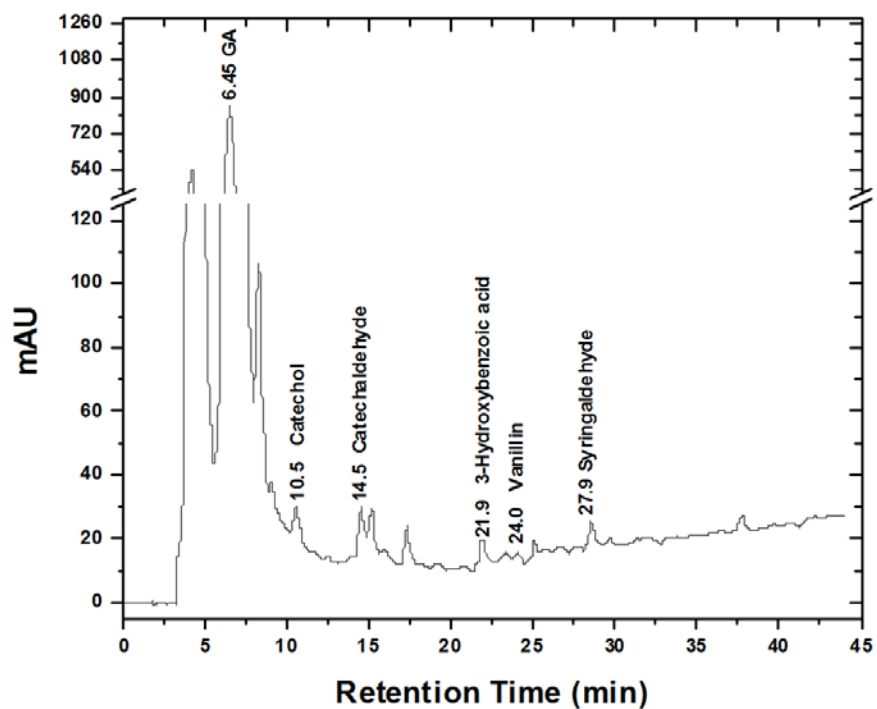


FIGURE S1. HPLC-UV chromatograms of polyphenolic rich maple syrup extract (PRMSE). Compounds were identified based on the peaks obtained from pure phenolic compounds in separate runs. The numbers on the peaks correspond to the retention time of pure phenolic compounds. (GA: gallic acid)

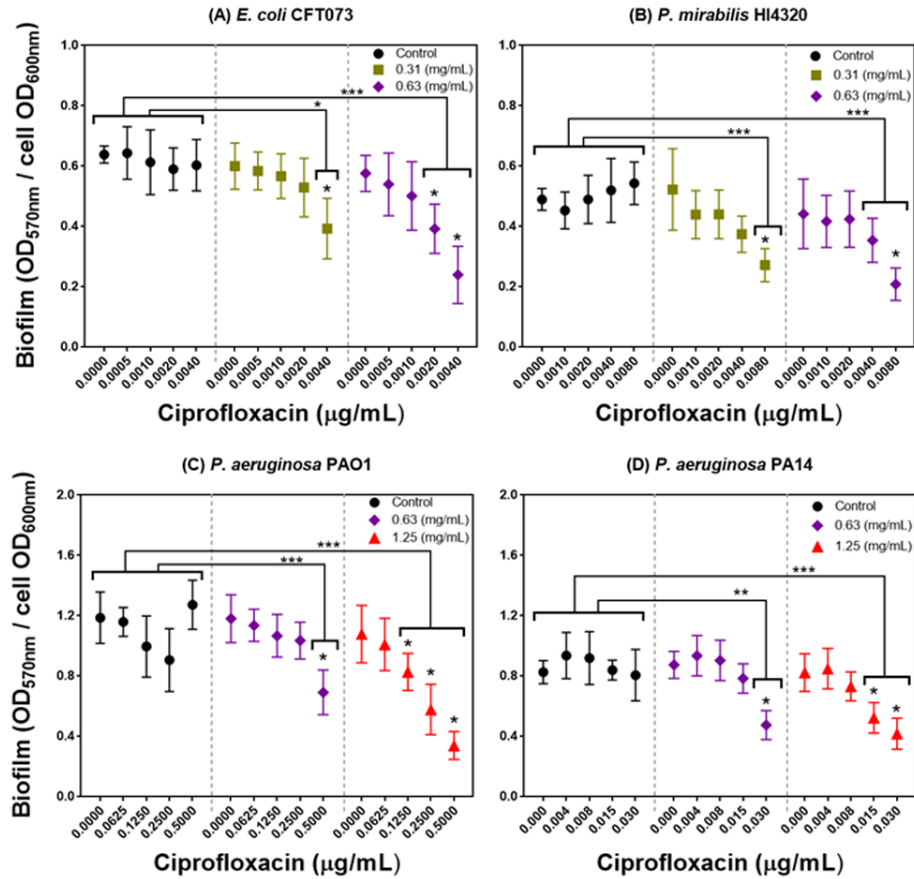


FIGURE S2. Effect of catechol with and without ciprofloxacin on biofilm formation of (A) *E. coli* CFT073, (B) *P. mirabilis* HI4320, (C) *P. aeruginosa* PAO1 and (D) *P. aeruginosa* PA14. The graph shows the normalized biofilm level (OD_{570nm}/cell OD_{600nm}) versus different sub-inhibitory concentrations of ciprofloxacin for each strain grown in LB medium (Control) or in LB medium amended with sub-inhibitory concentrations (0.31, 0.63 and 1.25 mg mL⁻¹) of catechol. Error bars are the standard deviation of data from three replications. Statistically significant differences are indicated for each sample treated with catechol + ciprofloxacin compared to control (sample treated with the corresponding concentration of ciprofloxacin only) (**: $p < 0.01$, ***: $p < 0.001$), and also for samples treated with catechol + ciprofloxacin compared to sample treated with the same concentration of catechol without ciprofloxacin (*: $p < 0.05$). Legend in each graph shows the concentration of catechol.

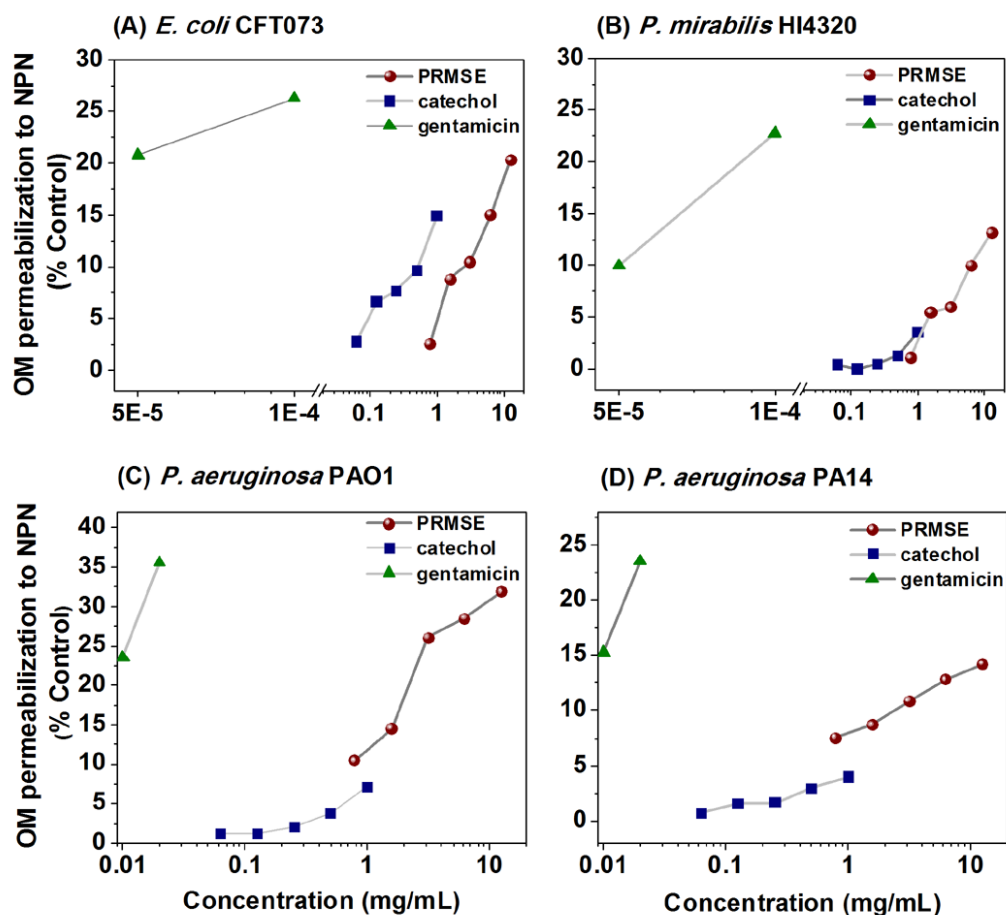


FIGURE S3. PRMSE, catechol and gentamicin-mediated NPN uptake in (A) *E. coli* CFT073, (B) *P. mirabilis* HI4320, (C) *P. aeruginosa* PAO1 and (D) *P. aeruginosa* PA14. Bacterial cells were pretreated with 1 mM *N*-ethylmaleimide (NEM) in 5mM HEPES buffer (pH 7.2) and incubated at room temperature in the presence or absence (control) of various sub-MICs of PRMSE, catechol and gentamicin. Enhanced uptake of NPN was measured by an increase in fluorescence (ex/em: 350nm/420nm) caused by partitioning of NPN into the hydrophobic interior of the outer membrane.

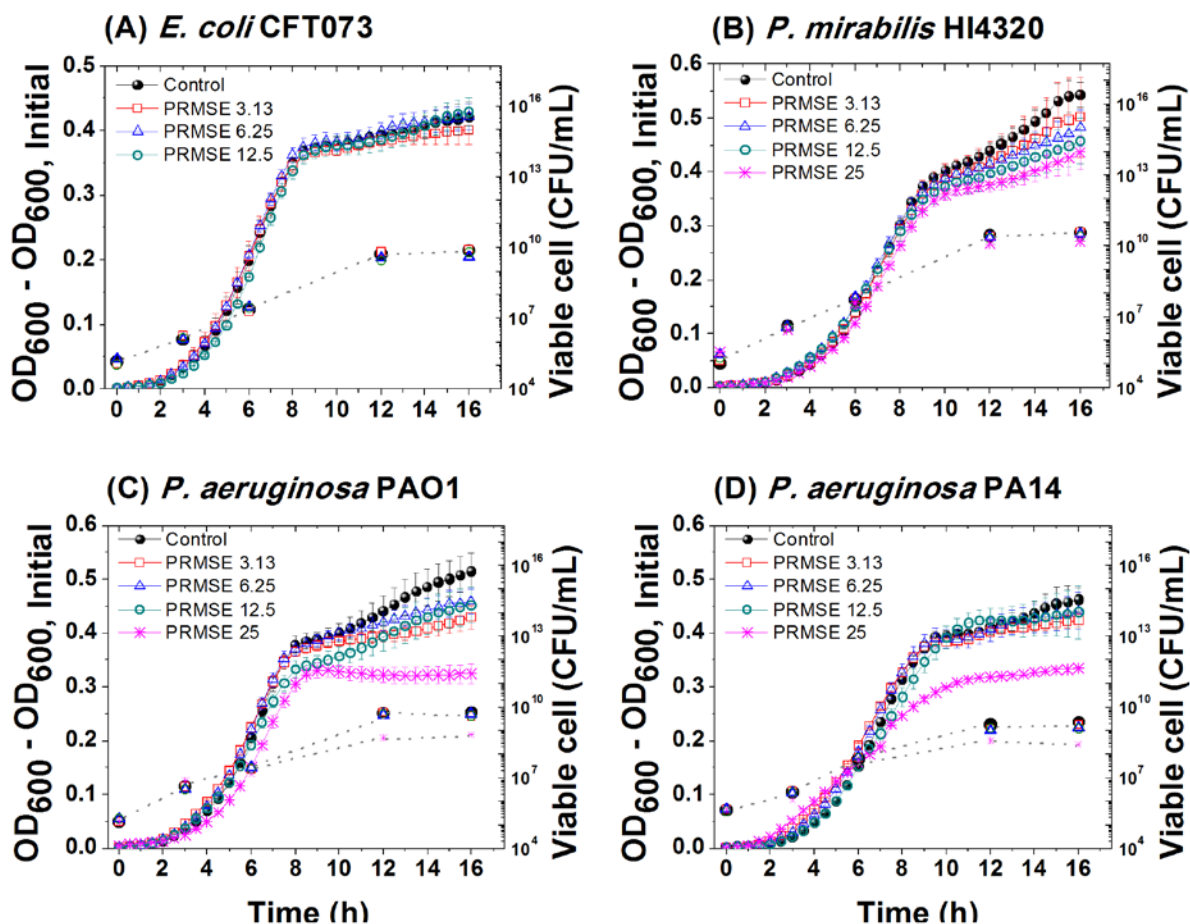


FIGURE S4. Growth curves in M9 minimal medium for (A) *E. coli* CFT073, (B) *P. mirabilis* HI4320, (C) *P. aeruginosa* PAO1 and (D) *P. aeruginosa* PA14, in the presence of PRMSE. Bacterial growth (OD_{600}) was monitored at 37°C for 16 h in M9 minimal medium. Error bars represent the standard deviation of values obtained from four replications of two independent experiments performed on different days. Viable cell population was monitored at same culture conditions and determined by serial dilution in PBS and plating on LB agar plates. Symbols with same color (for each concentration) and dotted lines represent the viable cell counts. Abbreviations: PRMSE. x , MS polyphenolic extract at x mg mL⁻¹ (e.g., PRMSE 25 indicates polyphenolic rich MS extract at 25mg mL⁻¹).

TABLE S1. Synergistic interactions of carbenicillin and PRMSE for growth inhibition

Interactions	<i>P. aeruginosa</i> strains	
	PAO1	PA14
MIC of carbenicillin ($\mu\text{g mL}^{-1}$)	64	128
FIC of carbenicillin	0.13	0.063
MIC of PRMSE (mg mL^{-1})	50	50
FIC of PRMSE	0.03	0.063
FICI	0.16	0.13
Synergistic	Yes	Yes