

Supplementary Table: Details of main treatments and accompanying controls with profiles of additives used in the assays. Results are shown as viabilities with SEM's in brackets based on at least 3 replicates; ranges are shown where appropriate.

Main treatments (MT + number, in bold) plus controls (Ref. to fig/table no. in main text in brackets)	Additives (final concentrations shown)						Inocula (0.5 McFarland standard)	Results Viability (log ₁₀ cfu mL ⁻¹)
	Catechin (μM)	Iron(II)/(III) (μM)	Copper(II) (μM)	VitaminC (μM)	EDTA (μM)	Catalase (μg mL ⁻¹)		
Buffer (fig 2)	^b Lambda						<i>S. aureus, E. coli, Ps.aeruginosa, P.mirabilis,</i>	<i>Sa, 7.66 (± 0.52); Ec, 7.08 (± 0.21) Psa, 7.83(± 0.21); Pm,7.18 (±1.08)</i>
(+)-Catechin alone (fig 2)	214						"	<i>Sa, 7.66 (± 0.52); Ec, 7.08 (± 0.21) Psa, 7.83(± 0.21); Pm,7.18 (±1.08)</i>
Ringer's pH: 3.5	Ringer's						"	<i>Sa, 7.63 (± 0.76); Ec, 7.77 (± 0.40) Psa, 7.92(± 0.55); Pm,7.87 (±0.43)</i>
pH: 5.5	Ringer's						"	<i>Sa, 7.61(± 0.82); Ec, 7.89 (± 0.83) Psa, 7.59(± 0.65); Pm,7.79 (±0.50)</i>
pH: 7.2	Ringer's						"	<i>Sa,7.65 (± 0.85); Ec, 7.60 (± 1.03) Psa, 8.01 (± 0.40);Pm,7.53 (±0.82)</i>
Cu(II): fresh (fig 2)	Ringer's		214				<i>S. aureus, E. coli, Ps.aeruginosa, P.mirabilis,</i>	<i>Sa, 5.92 (± 0.89); Ec, 3.09(± 0.56) Psa, 6.65(± 0.54); Pm,5.13 (±0.62)</i>
MT1. (+)-Catechin + copper(II) (fig 2)	214		214				<i>S. aureus, E. coli</i>	<i>Sa, 4.93 (± 0.66); Ec, 2.83(± 0.48) Psa, 4.11(± 0.73); Pm,4.11 (±0.73)</i>
Fe(II): fresh, (fig 3)	Ringer's	214 Fe(II) fresh					"	<i>Sa, 7.50 (± 0.82); Ec, 6.75 (± 0.62)</i>
heated,	Ringer's	214 Fe(II) heated					"	<i>Sa, 6.99 (± 0.37); Ec, 7.44 (± 0.62)</i>
autoclaved	Ringer's	214 Fe(II) autoclvd.					"	<i>Sa, 7.52 (± 0.55); Ec, 7.91 (± 0.76)</i>
Fe(III): fresh,	Ringer's	214 Fe(III) fresh					<i>S. aureus, E. coli</i>	<i>Sa, 7.33 (± 0.48); Ec, 7.87 (± 0.61)</i>
heated,	Ringer's	214 Fe(III) heated					"	<i>Sa, 7.49 (± 0.46); Ec, 7.77 (± 0.49)</i>
autoclaved	Ringer's	214 Fe(III) autoclvd.					"	<i>Sa, 7.99 (± 0.44); Ec, 8.03 (± 0.41)</i>
Cu(II): heated	Ringer's		214				"	<i>Sa, 6.01 (± 0.57); Ec, 4.25(± 0.38)</i>
MT2. (+)-Catechin + fresh, or heated or autoclaved Fe(II) or Fe(III)	214	214 Fe(II) fresh (fig3)					"	<i>Sa, 5.38 (± 0.53); Ec, 6.93 (± 0.50)</i>
	214	214 Fe(II) heated					"	<i>Sa, 7.51 (± 0.50); Ec, 8.05 (± 0.38)</i>
	214	214 Fe(II) autoclvd.					"	<i>Sa, 7.92 (± 0.42); Ec, 7.89 (± 0.61)</i>
	214	214 Fe(III) fresh					"	<i>Sa, 7.84 (± 0.68); Ec, 7.71 (± 0.48)</i>
	214	214 Fe(III) heated					"	<i>Sa, 7.22 (± 0.50); Ec, 8.64 (± 0.48)</i>

	214	214 Fe(III) autoclvd.					"	Sa, 7.23 (± 0.67); Ec, 4.53 (± 0.65)
Fe(II) + Cu(II) (fig 3)	Ringer's	214 Fe(II) fresh	214				"	Sa, 5.03 (± 0.65); Ec, 3.79 (± 0.70)
MT3. (+)-Catechin + Fe(II)+Cu(II) (fig 3)	214	214 Fe(II) fresh	214				"	Sa, 4.75 (± 0.45); Ec, 2.50(± 0.44)
Vit. C alone	Ringer's			1712			"	Sa 7.58 (± 0.56); Ec, 7.64 (± 0.44)
(+)-Catechin + vit. C	214			1712			"	Sa, 7.82 (± 0.34); Ec, 7.97 (± 0.51)
Cu(II) + vit. C alone (table 1)	Ringer's		214	428			"	Sa, 4.85 (± 0.52); Ec, 0 log ₁₀ units
			214	856			"	Sa, 4.37 (± 0.73); Ec, 0 log ₁₀ units
			214	1712			"	Sa, 2.41 (± 0.79); Ec, 0 log ₁₀ units
MT4. (+)-Catechin + Cu(II) + vit. C (table 1)	214		214	428			"	Sa, 3.77 (± 0.82); Ec, 0 log ₁₀ units
	214		214	856			"	Sa, 3.11 (± 0.12); Ec, 0 log ₁₀ units
	214		214	1712			"	Sa, 1.29 (± 0.48); Ec, 0 log ₁₀ units
Fe(II) + vit. C	Ringer's	214 Fe(II) fresh		856			"	Sa, 8.15 (± 0.63); Ec, 7.63 (± 0.15)
MT5. (+)-Catechin + Fe(II) + vit. C	214	214 Fe(II) fresh		856			"	Sa, 8.21 (± 1.11); Ec, 7.38 (± 0.55)
Fe(II) + Cu(II) + vit.C	Ringer's	214 Fe(II) fresh	214	856			"	Sa, 4.94 (± 1.02); Ec, 7.44 (± 0.59)
MT6. (+)-Catechin + Fe(II) + Cu(II) + vit. C (fig 3)	214	214 Fe(II) fresh	214	856			"	Sa, 4.76 (± 0.67); Ec, 2.60 (± 0.55)
EDTA alone	Ringer's				2140		"	Sa, 8.23 (± 0.41 ; Ec, 7.21 (± 0.51)
Fe(II) + Cu(II) + EDTA	Ringer's	214 Fe(II) fresh	214		535		"	Sa, 8.02 (± 0.33); Ec, 7.12 (± 0.49)
MT7. (+)-Catechin + Fe(II) + Cu(II) + EDTA	214	214 Fe(II) fresh	214		1070		"	Sa, 7.97 (± 0.36); Ec, 7.50 (± 0.42)
MT8. Cu(II) + catalase	Ringer's		214			0 - 200	"	Sa, 6.62-7.49 (± 0.33-1.02)
						0 - 600	"	Ec, 2.98-7.70 (± 0.25-1.09)

(fig 4) Catalase alone	Ringer's					600	"	<i>Sa</i> , 7.72 (± 0.33); <i>Ec</i> , 7.34 (± 0.45)
(+)-Catechin + catalase	214					600	"	<i>Sa</i> , 7.43 (± 0.49); <i>Ec</i> , 7.87 (± 0.48)
Fe(II) + Cu(II) +catalase	Ringer's	214 Fe(II) fresh	214			600	"	<i>Sa</i> , 8.16 (± 0.70); <i>Ec</i> , 6.60 (± 0.44)
Vit. C + catalase	Ringer's			856		600	"	<i>Sa</i> , 7.53 (± 0.48); <i>Ec</i> , 7.10 (± 0.62)
MT9. (+)-Catechin + Cu(II) + vit. C + catalase (fig 5)	214	214 Fe(II) fresh		856		0 - 200 0 - 600	" "	<i>Sa</i> , 1.84-7.74 (± 0.18-0.32) <i>Ec</i> , 2.12-7.27 (± 0.25-0.55)
Cu(II) + Vit.C + catalase	214					0 - 200 0 - 600	" "	<i>Sa</i> , 2.35-7.68 (± 0.08-1.10) <i>Ec</i> , 2.00-7.57 (± 0.31-0.46)
MT10. (+)-Catechin + Fe(II) + Cu(II) + catalase	Ringer's		214			600	"	<i>Sa</i> , 8.02 (± 0.35); <i>Ec</i> , 7.77 (± 0.53)
(+)-catechin + Fe(II) + catalase	214	214 Fe(II) fresh	214			600	"	<i>Sa</i> , 7.23 (± 0.42); <i>Ec</i> , 8.01 (± 0.53)
(+)-catechin + Cu(II) + catalase	214		214			600	"	<i>Sa</i> , 7.57 (± 0.48); <i>Ec</i> , 7.16 (± 0.49)

Fe(II) = aqueous iron(II) sulphate heptahydrate; **Fe(III)** = aq. iron(III) chloride solution; **Cu(II)** = aq. copper(II) sulphate pentahydrate; **EDTA** = aq. Ethylenediamine acetic acid disodium salt; **Catalase** = bovine liver catalase solution 50 mg mL⁻¹ with an activity of ca. 47 kU mg⁻¹ protein. **Inocula**: *Sa* = *S. aureus* = *Staphylococcus aureus* NCTC 06571, *Psa* = *Ps.aeruginosa* = *Pseudomonas aeruginosa* NCTC 950, *Pm* = *P.mirabilis* = *Proteus mirabilis* NCTC 7827, *Ec* = *E. coli* = *Escherichia coli* NCTC 14441

Notes on Methodology

The assay mixtures made up to produce the final concentrations shown above used following volumes and concentrations of solutions shown below:

Main treatments (MT + number, shown in bold) and controls:

Buffer: ^bAll assays featured 1000µL control samples of lambda buffer adjusted to pH 7.2 as a control.

Ringer's solution adjusted to different pH values: 1000 µL Ringer's solution adjusted to pH 3.5, or 5.5, or 7.2

(+)-Catechin alone: 330 µL 1000 µM (+)-catechin + 700 µL Ringer's solution

Cu(II) alone, fresh, heated: 330 µL Ringer's solution + 700 µL 472 µM Cu(II)SO₄

MT1: 330 µL 1000 µM (+)-catechin + 700 µL 472 µM Cu(II)SO₄

Fe(II) alone, fresh, heated, autoclaved: 330 µL Ringer's solution + 700 µL 472 µM Fe(II)SO₄ either freshly made, or freshly made and then heated, or freshly made and then autoclaved

Fe(III) alone, fresh, heated, autoclaved: 330 µL Ringer's solution + 700 µL 472 µM Fe(III)Cl₃ prepared as Fe(II) above

Fe(II) + Cu(II): 330 µL Ringer's solution + 350 µL 944 µM Fe(II)SO₄ + 350 µL 944 µM Cu(II)SO₄

MT2: 330 µL 1000 µM (+)-catechin + 700 µL 472 µM Fe(II)SO₄ or Fe(III)Cl₃, in each case either freshly made, or freshly made and then heated ,or freshly made and then autoclaved

MT3: 330 µL 1000 µM (+)-catechin + 350 µL 944 µM Fe(II)SO₄ + 350 µL 944 µM Cu(II)SO₄

Vitamin C alone: 330 µL Ringer's solution + 700 µL 1510 µM vit. C

(+)-Catechin + vit. C: 330 µL 1000 µM (+)-catechin + 700 µL 1510 µM vit. C

Cu(II) + vit. C alone: 330 µL Ringer's solution + 350 µL 944 µM Cu(II)SO₄ + 350 µL 1888 µM, or 3776 µM ,or 7552 µM vit. C respectively

MT4: 330 µL 1000 µM (+)-catechin + 350 µL 944 µM Cu(II)SO₄ + 350 µL 1888 µM, or 3776 µM, or 7552 µM vit. C respectively

Fe(II) + vit. C: 330 µL Ringer's solution + 350 µL 944 µM Fe(II)SO₄ + 350 µL 3776 µM vit. C

MT5: 330 µL 1000 µM (+)-catechin + 350 µL 944 µM Fe(II)SO₄ + 350 µL 3776 µM vit. C

Fe(II) + Cu(II) + vit. C: 330 µL Ringer's solution + 175 µL 1888 µM Fe(II)SO₄ + 175 µL 1888 µM Cu(II)SO₄ + 175 µL 3776 µM vit. C

MT6: 330 µL 1000 µM (+)-catechin + 175 µL 1888 µM Fe(II)SO₄ + 175 µL 1888 µM Cu(II)SO₄ + 175 µL 3776 µM vit. C

EDTA alone: 330 µL Ringer's solution + 700 µL 2140 µM EDTA solution

Fe(II) + Cu(II) + EDTA: 330 µL Ringer's solution + 175 µL 1888 µM Fe(II)SO₄ + 175 µL 1888 µM Cu(II)SO₄ + 175 µL 2140 µM EDTA

MT7: 330 µL 1000 µM (+)-catechin + 175 µL 1888 µM Fe(II)SO₄ + 175 µL 1888 µM Cu(II)SO₄ + 175 µL 2140 µM EDTA

MT8: 330 µL Ringer's solution + 700 µL 472 µM Cu(II)SO₄ + ^{*}catalase at concentrations specified

^{*}Catalase alone: 1000 µL Ringer's solution + catalase at concentration specified

(+)-Catechin + catalase: 330 µL 1000 µM (+)-catechin + 700 µL Ringer's solution + catalase at concentration specified

Fe(II) + Cu(II) + catalase: 330 µL Ringer's solution + 350 µL 944 µM Fe(II)SO₄ + 350 µL 944 µM Cu(II)SO₄ + catalase at concentration specified

Vit. C + catalase: 650 µL Ringer's solution + 350 µL 3776 µM vit. C + catalase at concentration specified

MT9: 330 µL 1000 µM (+)-catechin + 350 µL 944 µM Cu(II)SO₄ + 350 µL 3776 µM vit. C + catalase at concentrations specified

Cu(II) + vit. C + catalase: 650 µL Ringer's solution + 350 µL 3776 µM vit. C + catalase at concentrations specified

MT10: 330 µL 1000 µM (+)-catechin + 350 µL 944 µM Fe(II)SO₄ + 350 µL 944 µM Cu(II)SO₄ + catalase at concentration specified

(+)-Catechin + Fe(II) + catalase: 330 µL 1000 µM (+)-catechin + 700 µL 472 µM Fe(II)SO₄ + catalase at concentration specified

(+)-Catechin + Cu(II) + catalase: 330 µL 1000 µM (+)-catechin + 700 µL 472 µM Cu(II)SO₄ + catalase at concentration specified

^{*}Catalase was added immediately prior to addition of inoculum

Full details of the assay procedure is described in 'Antimicrobial activity of (+)-catechin and transition metal ion combinations' in the Materials & Methods section of main article