

Minimal Inhibitory Concentration of a Novel Plant Extract on Growth of Bacterial Isolates with Veterinary Importance

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BACKGROUND: Plant antimicrobial function is based on a different mechanism than antibiotics or chemical sanitizers; however, commercially available plant-based bacteriostatic products have failed to achieve broad spectrum potency without formulation, user preference, or toxicity problems.

The Grazix feed supplement (LiveLeaf, USA) is a novel extract of green tea (*Camellia sinensis*) leaves and pomegranate (*Punica granatu*) fruit. Using a proprietary extraction process, plant-derived polyphenols are combined with natural metabolites to deliver the immune benefits effectively at very low concentration. It has the potential to provide immune modulation to pathogens typically encountered in commercial porcine farms.

OBJECTIVE: To determine the minimal inhibitory concentration (MIC) of Grazix solution for select Gram-positive and Gram-negative bacteria with veterinary importance

METHODS: GRAZIX was diluted in sterile distilled water to achieve concentrations of 500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.9, 1.95, 0.98, and 0.49 μ L GRAZIX per mL of water. Strains of Gram-negative (*Bordetella bronchiseptica*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar Typhimurium*) and Gram-positive (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*) bacteria were isolated from different pathological samples originating from swine and poultry in a veterinary diagnostic bacteriological laboratory (Veterinary Diagnostic Directorate, NFC SO, and Department of Microbiology and Infectious Diseases, SZIU-FVS, Budapest, Hungary) and cultured in Trypticase Soy Broth (TSB) for several hours until the resulting culture's viable bacterial cell count was between 10^4 and 10^5 CFU/mL.

The GRAZIX dilutions were added to tubes containing Mueller Hinton Broth followed by the addition of 10 μ L of bacterial suspension; as a control, sterile water was used in place of dilutions of the plant extract. This mixture was incubated at 37° C for 24 hours and then the degree of inhibition of bacterial growth was evaluated visually, with the MIC values determined by either growth visible (the presence of turbidity in the tube) or no growth visible (no turbidity).

RESULTS: All controls exhibited bacterial growth (Figure 1); all but two bacterial isolates were inhibited by the GRAZIX solutions diluted to 3.9 μ L/mL or 7.8 μ L/mL (Table 1). One Gram-positive and one Gram-negative isolate were not inhibited until 15.6 μ L/mL and 31.3 μ L/mL solutions, respectively, were applied.

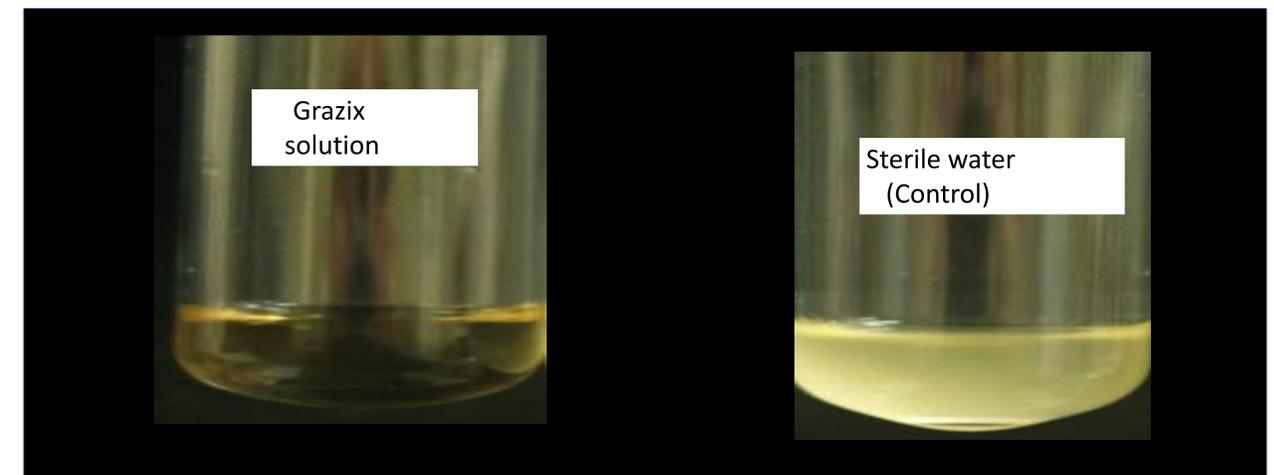


Figure 1. Total inhibition of *E. coli* by Grazix solution (left); no inhibition in sterile water as control (right).

Examined Material	Bacterium Species (μ L/ml) (μ g/ml or ppm)	Concentration (dry plant wt. equiv)										
		500	250	125	62.5	31.3	15.6	7.81	3.9	1.95	0.98	0.49
		750	375	188	94	47	23	12	6	3	1.5	0.74
110909-HT Grazix (2500ppm stock) Source: Szent Istvan University Budapest	<i>Escherichia coli</i>	—	—	—	—	—	—	—	—	+	+	+
	<i>Salmonella enterica st. Typh.</i>	—	—	—	—	—	—	—	+	+	+	+
	<i>Staphylococcus aureus</i>	—	—	—	—	—	—	—	—	+	+	+
	(MRSA) <i>Staphylococcus aureus</i>	—	—	—	—	—	—	—	—	+	+	+
	<i>Pseudomonas aeruginosa</i>	—	—	—	—	—	—	—	+	+	+	+
	<i>Listeria monocytogenes</i>	—	—	—	—	—	—	+	+	+	+	+
	<i>Pasteurella multocida</i>	—	—	—	—	—	—	—	+	+	+	+
	<i>Proteus vulgaris</i>	—	—	—	—	—	+	+	+	+	+	+
	<i>Klebsiella pneumonia</i>	—	—	—	—	—	—	—	—	+	+	+
	<i>Bacillus cerus</i>	—	—	—	—	—	—	—	—	+	+	+
<i>Bordetella brochiseptica</i>	—	—	—	—	—	—	—	—	+	+	+	

— MIC falls within first + — No growth in broth culture (no turbidity) + Visible growth in broth culture (turbidity)

CONCLUSION: These results demonstrate that the novel plant extract, Grazix feed supplement, has antibacterial activity against pathogens of veterinary importance.