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## Minimal inhibitory concentration of a novel plant extract on growth of bacterial isolates with veterinary importance

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**Abstract** LiveLeaf GRAZIX is an extract of green tea (*Camellia sinensis*) and pomegranate (*Punicagranatu*). Plant antimicrobial function is based on a different mechanism than antibiotics or chemical sanitizers. Such natural plant compounds have a long history of use as bacterial inhibitors. However, commercially available plant-based bacteriostat products have remained expensive and have failed to achieve broad spectrum potency without formulation, user preference or toxicity problems. The objective of the present study was to determine MIC of a novel plant extract, GRAZIX, as feed additive against Gram-negative and Gram-positive bacteria by using serial dilutions of the extract. The plant extract, 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  $\mu\text{L}$  of 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 samples originating from the swine and poultry with diseases submitted to a veterinary diagnostic bacteriological laboratory (Veterinary Diagnostic Directorate, NFCSO, and Department of Microbiology and Infectious Diseases, SZIU-FVS, Budapest, Hungary). The samples were 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  $\mu\text{L}$  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). All controls exhibited bacterial growth; all but two bacterial isolates were inhibited by the GRAZIX solutions diluted to 3.9  $\mu\text{L}/\text{mL}$  or 7.8  $\mu\text{L}/\text{mL}$ . One Gram-positive and one Gram-negative isolate were not inhibited until 15.6  $\mu\text{L}/\text{mL}$  and 31.3  $\mu\text{L}/\text{mL}$  solutions, respectively, were applied. The results demonstrated that the novel plant extract, GRAZIX, has antibacterial activity against various pathogens of veterinary importance.

**Keywords** phytobiotics; feed additive; plant extract; Grazix; bacteria; minimal inhibitory concentration