TITLE: ANTIBIOFILM POTENTIAL, TOXICITY *IN VIVO* AND POLYPHENOLIC PROFILE OF *Eugenia brasiliensis* (GRUMIXAMA), AN UNEXPLORED BRAZILIAN NATIVE FRUIT.

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ABSTRACT:

Eugenia brasiliensis Lam. (EB) commonly known as "Grumixama'' is a Brazilian native tree growing in the Atlantic rainforest whose seeds and leaves have shown a promising antifungal and antibiofilm activity against *Candida albicans.* EB fruit pulp, however, has never been studied for these properties against pathogenic bacteria and toxicity *in vivo*. Herein, we evaluate the antibiofilm potential, toxicity *in vivo* and describe the polyphenolic composition of EB pulp extract. The pulp was collected in a local farm (S 23º 23', W 45º 39') in the city of Paraibuna, SP, Brazil (voucher #ESA056895 and CNPq #010907/2014-9). The hydroethanolic extract (80:20, v/v) from EB pulp was obtained and its polyphenolic profile was determined by Liquid Chromatography coupled to Mass Spectrometry (LC-MS/MS). The Minimal Inhibitory/Bactericidal Concentration (MIC/MBC) of the extract was determined against methicillin-susceptible and –resistant *Staphylococcus aureus* (MSSA/MRSA), *Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans,* and *Lactobacillus acidophilus.* A maximum MIC threshold of up to 100 µg/mL was selected to proceed with the analysis of mature biofilms. Finally, the acute toxic effects of EB extract and LD_{50} were determined *in vivo* using the *Galleria mellonella* larvae model. The data were analyzed by one-way ANOVA with Tukey's post-hoc test (α=0.05). The polyphenols catechin, ellagitannin, flavonols and anthocyanins were found in EB extract composition. The MIC range of the EB extract ranged from 62.5 to 500 µg/mL while MBC values were found to be above 500 µg/mL. It was selected the *L. acidophilus* and *S. aureus* (MIC<100 µg/mL) to biofilm growth. EB extract significantly reduced *L. acidophilus* biofilm formation ($P<0.05$). However, EB extract not reduced significantly *S. aureus* biofilm cells as compared to control group ($P>0.05$). At the highest dose tested (10 g/kg), EB extract killed only 1% of larvae population as compared with saline-treated control ($P>0.05$) and it was not possible to found the lethal doses able to kill 50% of the larvae. Thus, EB extract affected *L. acidophilus* biofilm formation possibly due the presence of phenolic compounds and showed low toxicity *in vivo*. Our findings open new perspectives concerning the application of EB extract as a functional food, pharmaceutical lead and/or as an agribusiness commodity.

Keywords: Biofilm, *Eugenia brasiliensis*, Native Fruit, *Galleria mellonella*.

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