TITLE: ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF *VARRONIA CURASSAVICA* AGAINST *XANTHOMONAS CAMPESTRIS* PV AND EFFECT ON THE INTEGRITY OF THE CYTOPLASMIC MEMBRANE.

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ABSTRACT:

In this study we investigated the antimicrobial characteristics, chemical composition and mechanism of action of the best essential oil (OE) of Varronia curassavica on the target phytopathogen *Xanthomonas campestris pv.campestris* (*Xcc*). GC-MS analysis identified 84 compounds among the OEs of the seven genotypes of V. curassavica with trans-caryophyllene and α-humulene as major major compounds. The seven genotypes of V.curassavica OEs (VCUR-002, -104, -202, -302, -303, -601, -701) exhibited strong (MIC ≤0.5 mg / mL) to moderate (MIC ≤1 , 0 mg / mL) antimicrobial activity on *Xcc*, being genotypes VCUR-202 and VCUR-302 with lower MIC values 0.5 mg / mL. The curve of the time-kill study showed that both OE VCUR-202 (2x, 1x, 1 / 2x MIC) and VCUR-302 and (2x, 1x MIC) inhibited 100% of the cell growth of *Xcc*-629IBSBF after 24 hours exposure. These data can be confirmed by the formation of UFC.mL-1. In addition, in this study the mode of action of OES-VCUR-202, -303 against *Xcc* was investigated using propidium iodide (pI), fluorescence electron microscopy, nuclear cell material extravasation by absorption rate UV260nm and UV280nm, electrical conductivity and variation of extracellular pH, as well as change in membrane potential (PM) using the Rhodamine 123 (Rh123) probe. After exposure to OE-VCUR 202 (2x, 1x, 1/2 x MIC) and VCUR-302 (2x, 1x MIC) for 6 hours, there was decrease in cell viability, increase in pI fluorescence, and release of nucleic acids. For both OEs, a considered increase (2x MIC and 1x MIC) was observed in the variation in electrical extracellular conductivity (CDE), relative electric conductivity (CDR) and extracellular pH of *Xcc*. Rh123 Values represented by Mean Fluorescence Intensity (Excitation485nm / Emission530nm) by the spectrofluorimetric technique showed that viable bacteria labeled with Rh123 could replicate. The accumulation of Rh123 in these cells did not induce a high Average Fluorescence Intensity (IMF). Thus, this study indicates that OEs VCUR-202, -302 of V. curassavica cause damage to the cytoplasmic cell membrane reducing the antimicrobial viability being a natural product option in the biological control against *Xcc*.

Keywords: Antimicrobial activity, essential oils, *xanthomonas campestris* pv, cytoplasmic membrane.

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