The search for alternative compounds to develop a rational use in food conservation in order to answer to a customers’ demand for safer, more natural and resistant products has attracted a massive scientific interest on the investigation of various medicinal and aromatic plants and condiments due to their high potential as natural agents. In special, the essential oils have received a great attention because of their proven antimicrobial property. The objective of this study was to identify the chemical composition and to evaluate antimicrobial activity of the essential oil from leaves of *Croton rhamnifoliioides* against pathogenic bacteria of interest in food. The identification and quantification of the essential oil compounds was done through a gas chromatography–mass spectrometry (CG-MS). The Minimum Inhibitory Concentration (MIC) was determined through the technique of macrodilution. Eight components were identified: 1.8-cineole (46.32%) was the major compound, followed by 1-felandrene (16.70%), *p*-cymene (10.21%), sabinene (8.14%) and trans-caryophyllene (4.81%). The essential oil had antibacterial activity against *Aeromonas hydrophila*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus*, with Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations ranging within 2.5–20 and 5–40 µL.mL⁻¹, respectively. These results demonstrate the antibacterial potential of the essential oil from leaves of *C. rhamnifoliioides*, suggesting the possibility of its application in the inhibition of growth and survival of food pathogens.