

TITLE: RED BLOOD CELL HEMOLYTIC ASSAY – AN ALTERNATIVE TO ASSESS CYTOTOXICITY OF ESSENTIAL OILS WITH POTENTIAL ANTIMICROBIAL ACITIVITY

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Abstract:

The rising of microbial resistance to antibiotics have led the pharmaceutical industry to search for different sources to develop new potential antimicrobial drugs, and for that plants essential oils may be a useful alternative. However, for new pharmaceutical realising it is necessary to determine the product's efficacy and safety through pharmacological and toxicological analysis. Given the growing ethical questioning regarding the use of animal testing in the analysis of toxicity research and *in vitro* assay validation is a viable alternative to animal replacement. In this sense, this study evaluated the hemolytic activity of four essential oils known known to have in their compositions components with antimicrobial potential and extracted from *Syzygium jambolanum*, *Lippia gracilis*, *Myracrodruon urundeuva* and *Bixa orellana* plants, by applying the red blood cell hemolytic assay, since it is a simple technique, low cost, and effective to screening natural extracts cytotoxicity. With this purpose, a hemolytic assay was carried out in micro-centrifuge tubes containing 10% sheep erythrocytes suspension using samples in different concentrations previously established, at 37 °C/60 min. The hemolysis percentage was determined by spectrophotometer reading at 540 nm. All assays were performed in triplicate. The results showed values below 10% of hemolysis indicating that the essential oils did not show any hemolytic activity against the erythrocytes suspension, and thus no toxicity was observed. These promising results are significant for the use of the substances as well as to confirm the relevance of the test, since it is a test commonly used in toxicological analyses of different types of materials, and although there are some limitations related to its applicability, the red blood cell hemolytic assay has presented numerous positive points and advantages over other techniques.

Keywords: alternative methods; essential oils; antimicrobial drug resistance; hemolytic activity; *in vitro* toxicity.

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