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## Isolation of a trypanocidal sesquiterpene lactone from *Stevia gilliesii* by chromatographic techniques

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Chagas disease is an illness caused by the protozoan *Trypanosoma cruzi* which affects about 6-7 million people worldwide. Sesquiterpene lactones (STL) are a large group of secondary metabolites mostly found among Asteraceae family plants. Sesquiterpene lactones present a wide range of biological activities such as anti-inflammatory, cytotoxic, antiplasmodial and antitrypanosomal, among others. In a previous work, we have evaluated the effect of a group of STLs against T. cruzi epimastigotes and their cytotoxicity against Vero cells. *Eupahakonenin B* (EKB), isolated from *Stevia gilliesii* var. gilliesii Hook. and Arn. (Asteraceae) has shown promising trypanocidal activity and selectivity index (IC50=0.78 μM; CC50=363.72 μM; SI=466). In this work we describe the isolation of EKB by chromatographic methods in order to assess its trypanocidal activity against infective and intracellular forms of T. cruzi. Dried aerial parts of *S. gilliesii* var. gilliesii were extracted twice with dichloromethane. Dichloromethane extract was partitioned between hexane and a water/ethanol mixture and fractionated by column chromatography using silicagel and dichloromethane/ethyl acetate (1:2) as mobile phase. Eluted fractions were analyzed by thin layer chromatography (sp: silica gel 60 F254, mp: Dichloromethane/ethyl acetate 1:2). EKB containing fractions were joined and taken to dryness to obtain the compound as a greenish gum. EKB purity was analyzed by HPLC. The effect of EKB on bloodstream infective form of T. cruzi was assayed by counting remaining living parasites in a Neubauer chamber and the effect of EKB on intracellular forms of T. cruzi was assayed using β-galactosidase transfected parasites as previously described. The sesquiterpene lactone EKB appeared as a blue spot on TLC after spraying the plate with anisaldehyde-sulphuric acid (Figure 2). The STL was purified and isolated by chromatographic techniques (96% purity) and identified by spectroscopic methods (Figure 2). This STL showed activity and selectivity on trypomastigote (IC50=30 μM; SI=11) and amastigote (IC50=29.9 μM; SI=12) forms of T. cruzi. The mechanism of action of EKB and its *in vivo* activity will be evaluated.

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