Reviewer's report

Title: In vitro cytocidal effects of the essential oil from Croton cajucara (red sacaca) and its major constituent 7- hydroxycalamenene against Leishmania chagasi

Version: 5 Date: 16 August 2013

Reviewer: Tania Ueda-Nakamura

Reviewer's report:

Comments to the Authors’ response:

Reply 1: Photomicrographs were uploaded in better resolution
Comment: OK.

Reply 2: The difference between cell lysate gel and supernatant gel occurred because they were run in different electrophoresis apparatus. However, molecular weight standards were used to ensure the identification of the bands. Figure 3 was modified accordingly.
Comment: OK.

Reply 3: The following sentence was included in Figure 4 legend: “(MØ) macrophages; (P) parasites; (PtMØ) pre-treated macrophages; (PtP) pretreated parasites; (IMØ) infected macrophages; (Post-IMØ) post-treated infected macrophages.”
Comment: OK.

Minor Essential Revisions

Reply 4: As suggested by Referee 1, the word "antimicrobial" was replaced by "antileishmanial".
Comment: The word “antimicrobial” or “antileishmanial” should be suppressed, because if you say: “In vitro activity of ….. against L. chagasi promastigotes…” we can conclude that there is an antileishmanial activity.

Reply 5: In order to evaluate the effect of essential oil on peritoneal macrophages, these cells were treated 20 min prior to the interaction with the essential oil. Based on previous works (Rosa et al. 2003; Ueda-Nakamura et al., 2006; Santin et al. 2009), we believed that the 7-hydroxycalamenene-rich essential oil could promote macrophage stimulation and consequently enhance their killing mechanism. In the present work, we observed that pretreatment of macrophages with 250 and 125 µg/mL of essential oil reduced the number of adherent and internalized parasites (Association indices) in 30.0 and 9.6%, respectively.
Comment: Perhaps the sentence: “Association indices 30.6 and 9.6% lower were observed upon pretreatment of macrophages with 250 and 125 µg/mL of the essential oil, respectively.” could be replaced by “In the present work, we observed that pretreatment of macrophages with 250 and 125 µg/mL of essential oil reduced the number of adherent and internalized parasites (Association Indices) in 30.0 and 9.6%, respectively” because it is more understandable.

Major Compulsory Revisions

Reply 6: E-64 and phenanthroline are protease inhibitors that specifically target cysteine peptidases and metallopeptidases, respectively. Those inhibitors were used to identify the classes of peptidases that were being affected by the essential oil. E-64 was able to inhibit the peptidase activity of the 54- and the 41.5-kDa peptidases, while phenanthroline completely inhibited peptidase activity.

Comment: Considering that the samples in the presence /absence of protease inhibitors were not showed (in the gel), I suggest the inclusion of the information that “E-64 was able to inhibit the peptidase activity of the 54- and the 41.5-kDa peptidases” in the text. Authors state that in the supernatant “As demonstrated by band intensity, one-hour exposure to the essential oil increased the activity of only one group of cysteine peptidases of 54 kDa (Fig. 3).” But in my opinion it seems that the difference of 54 kDa peptidase activity between treated and untreated parasites is not so significant. In order to confirm and conclude, the density of bands should be determined.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests'