Table 1
Comparison of the results of biochemical tests between different strains of *Leptotrichia trevisanii*.

Test	Our case	Tee et al.[1]	Cooreman et al.[6]	Higurashi et al.,[2] case 1	Higurashi et al.,[2] case 2
Catalase	–	+	–	NT	NT
Oxidase	–	–	–	NT	NT
Urea	–	–	NT	–	–
Indole	–	–	–	–	–
αGAL	+	–	NT	+	–
βGAL	+	NT	+	NT	NT
αGLU	+	+	NT	+	–
βGLU	+	+	NT	+	–
βNAG	+	+	NT	–	–
PAL	+	–	NT	–	–
Esculin	NT	NT	NT	+	+

+: positive; – negative; NT: not tested.

positive,[3] the same as Schmitt et al., who did so in a period of 48 h.[5] In our case, it allowed us to establish the aetiology of the bacteremia within 18 h after the passage of positive blood cultures to solid culture media.

The sensitivity patterns for *Leptotrichia* spp. are not defined, although it has been described as sensitive to most antimicrobials.[1,2,6,7] There is not enough experience to establish a treatment of choice. In the reviewed literature several therapeutic regimens were implemented based on the sensitivity study, with resolution of the clinical picture in all cases. Our patient was treated with piperacillin-tazobactam, the blood cultures being negative on the tenth day after treatment and with resolution of mucositis on day 15. However, Martín-Gutiérrez et al. did not observe clinical improvement using piperacillin-tazobactam, changing the treatment to meropenem.[3]

The use of levofloxacin is inadequate in these cases, since *Leptotrichia trevisanii* shows *in vitro* resistance to fluoroquinolones.[2,6] Schrimsher et al. described 3 clinical cases in which levofloxacin was used as an empirical treatment in febrile neutropenia, which did not prevent the development of bacteremia by *Leptotrichia*.[8] Therefore, it is important that patients with febrile neutropenia and mucositis, more prone to infections by anaerobic micro-organisms, are treated with anaerobicidal antimicrobials.[7,8]

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Activity of *Artemisia annua* infusions on epimastigotes of *Trypanosoma cruzi*☆

Actividad de infusiones de Artemisia annua *sobre epimastigotes de* Trypanosoma cruzi

Chagas disease is an anthropozoonosis caused by the flagellate protozoan *Trypanosoma cruzi (T. cruzi)*.[1] Currently, the treatment against *T. cruzi* is restricted to two drugs of limited effectiveness and high toxicity: benznidazole and nifurtimox.[2] Therefore, it is necessary to find new therapeutic tools. The use of natural products such as *Artemisia annua (A. annua)* represents a novel choice; its active compound is artemisinin, a sesquiterpenoid lactone that crosses the biological membranes.[3] This study evaluated the effect of the *A. annua* infusion on epimastigotes of *T. cruzi*.

Epimastigotes of *T. cruzi* were used (isolated: RHO/Ve/03/RG1 and CHHP). For the preparation of the infusions, dry and crushed *A. annua* leaves from 2 different origins were used: from plants grown in Cumaná (Venezuela) and in Luxembourg. The infusions were prepared at concentrations of 0.4, 0.6, 0.8, 1.0, 2.0 and 3.0% m/v of dry leaves of *A. annua* in the *Liver Infusion Tryptose* (LIT) culture medium.[4] The experiments were performed by adjusting the cultures to a cellular density of 2×10^6 parasites/ml, in the various, previously described concentrations of the *A. annua* infusion. The trials were performed in triplicate and a control culture was included. The cellular viability of the epimastigotes of *T. cruzi*, was determined by the trypan blue dye exclusion method.[5] In order

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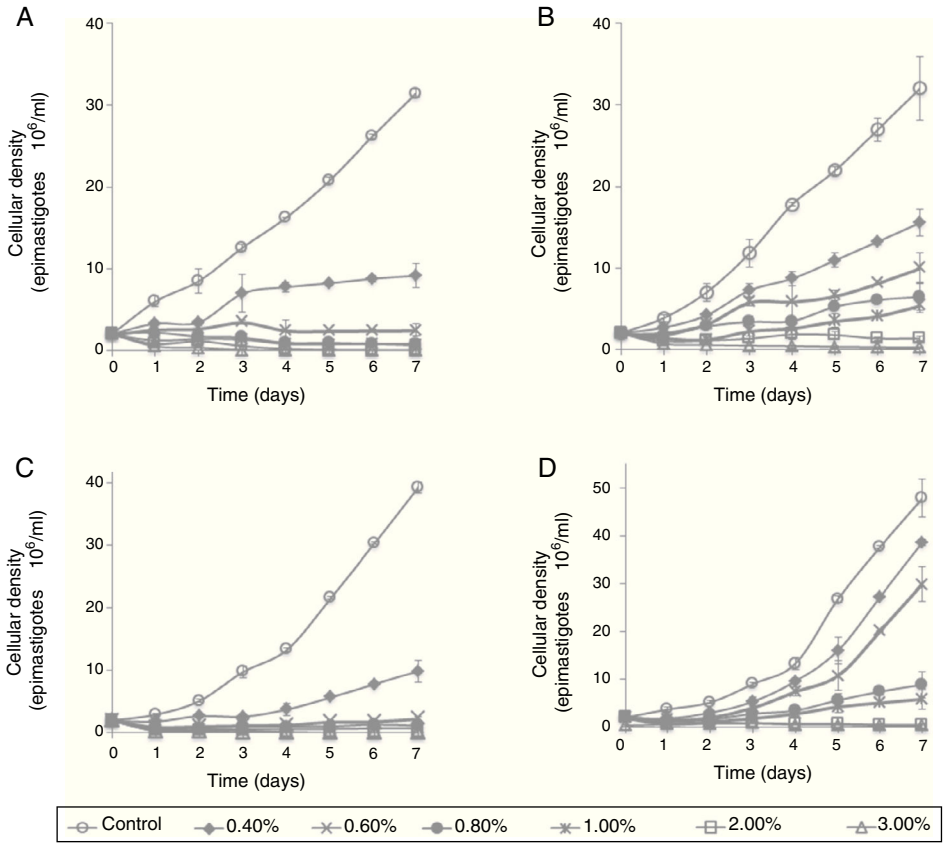

Fig. 1. Activity of *Artemisia annua* infusion on epimastigotes of *Trypanosoma cruzi*. (A) Treatment using plant grown in Luxembourg on isolated CHHP. (B) Treatment using plant grown in Cumaná, Venezuela on isolated CHHP. (C) Treatment using plant grown in Luxembourg on isolated RG1. (D) Treatment using plant grown in Cumaná, Venezuela on isolated RG1.

to determine the effect of the *A. annua* infusion concentration on the growth of epimastigotes of *T. cruzi*, a multivariate analysis of variance was used.

The infusions of *A. annua* from Cumaná and Luxembourg had, at all concentrations tested, a dose-dependent antiproliferative effect on both isolates of *T. cruzi* studied, compared to the control culture. At the end of treatment (7th day) it was found that the concentrations that produced a greater inhibition of the growth were 2% and 3%; likewise, it was observed that the infusions of *A. annua* from Luxembourg had a more potent effect on parasite inhibition compared to infusions of plants grown in Cumaná, Venezuela (Fig. 1). This fact was confirmed by evaluating the minimum inhibitory concentration (MIC) in the various experiments, finding that the MIC of the Luxembourg-RG1 treatment was 2.6 times lower than that of the Cumaná-RG1 treatment; meanwhile the MIC of the Luxembourg-CHHP treatment was two times lower than that of the Cumaná-CHHP treatment. The multivariate analysis of variance revealed that the effect of infusions on the cellular density of epimastigotes of *T. cruzi* was exerted in a highly significant manner ($p < 0.001$) depending on the type of infusion, concentration and time of exposure, and not significant for the different isolates evaluated. Regarding the incubation time of the cultures in the *A. annua* infusion, significant differences were found between days 0, 1, 2, 3 and 5 compared to day 7, indicating that the first days of treatment are key in the active inhibition of the epimastigotes of *T. cruzi*. During the course of treatment, the epimastigotes gradually lost their mobility, changing their typical fusiform morphology to an elongated and thin or rounded form, without flagella.

This is the first study demonstrating the antiproliferative effect of *A. annua* on Venezuelan isolates of *T. cruzi*. Other authors have tested the antiproliferative effects of purified artemisinin on *T. cruzi* and *T. brucei rhodesiense*.[6] The greater antiproliferative effect of the infusion prepared with the plant from Luxembourg could be due to the different production of active compounds and essential oils of both plants, which varies depending on the geographical origin of the plant and on the temperature.[7] High ambient temperatures, such as is the case in Cumaná, Venezuela, produce a decrease in artemisinin concentrations.[8] Also, the fact that *A. annua* infusions exerted their antiproliferative effect during the 7 days of treatment suggests that the other compounds present in the infusion could act synergistically as a combination therapy to perform their antiproliferative action.[9]

The results obtained in this study open up new research perspectives on the trypanocidal action of *A. annua* on *T. cruzi*, and offer a therapeutic alternative to be studied for the treatment of infection by this parasite.

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