



3rd International Electronic Conference on Medicinal Chemistry

1-30 November 2017

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by



pharmaceuticals

Search of Trypanosomicidal Active Principles by Metabolomic-guided Fractionation in *Baccharis trimera*

Javier Nargoli¹*, Javier Varela¹, Hugo Cerecetto^{1, 2} and Mercedes González¹

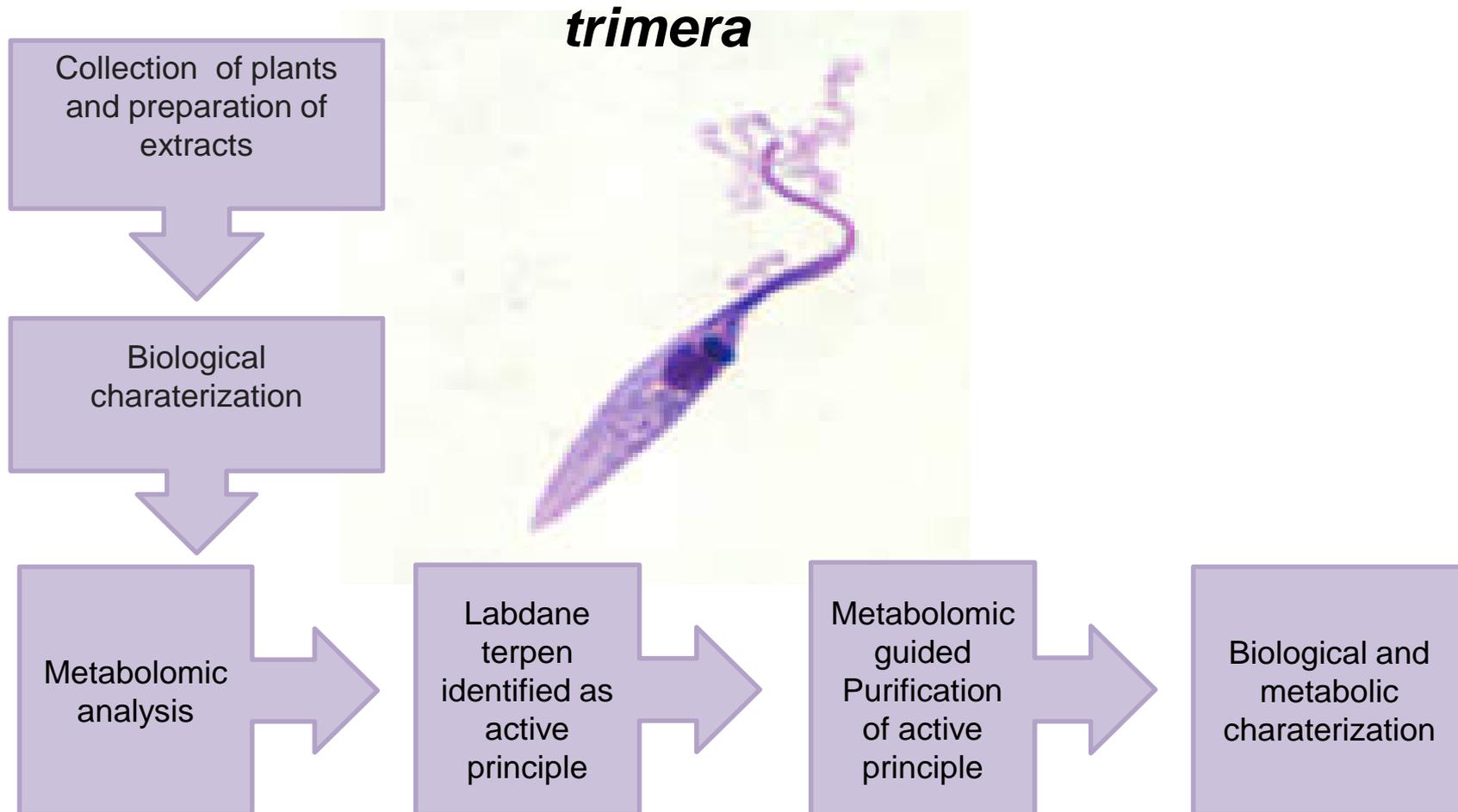
¹ Grupo de Química Medicinal, Facultad de Ciencias Universidad de la República, Iguá. 4225, Montevideo, 11400.Uruguay;

² Área de Radiofarmacia, Centro de Investigaciones Nucleares, Facultad de Ciencias, Universidad de la República. Uruguay.

* Corresponding author: javiernargoli@gmail.com



Search of Trypanosomicidal Active Principles by Metabolomic-guided Fractionation in *Baccharis trimera*



Abstract:

The American Trypanosomiasis, also known as Chagas disease is caused by *Trypanosoma cruzi*, a protozoan of the Trypanosomatidae Family. It is a zoonotic endemic that affects approximately 6-8 million people, it is considered a neglected diseases making it not attractive for pharmaceutical industries. Currently available treatments use the drugs a nitrofurfurylidene-amino (Nifurtimox) and a nitroimidazole acetamide (Benznidazole). Both are not completely effective against the disease. To overcome these problems we are using natural products combined with nuclear magnetic resonance based metabolomic analysis. We could identify the responsible compound of the trypanosomicidal activity in *Baccharis trimera*, this being a diterpene of the labdane type containing an aldehyde, agreeing with results obtained by the group previously where metabolites of the same nature had been described with trypanosomicidal activity in *Aristeguieta glutinosa*. In the present work the *Baccharis trimera* fractionation oriented to verified the above compound is performed using gradient of polar disolvents extractions, and the biological activity of the fractions obtained in the process is monitored by *in vitro* assays in the epimastigote form of *T. cruzi*, Tulahuen 2 strain and the ¹H NMR metabolomic characterization. The results obtained confirm that the ethyl acetate *Baccharis trimera* fraction has an important anti- *T. cruzi* activity, and besides that the aldehyde-diterpene is not the only metabolite with biological activity present in the plant, so we can also infer that we are facing a synergistic effect.

Keywords: Natural products, chagas disease, *t-cruzi*, trypanosomiasis, *Baccharis*



Introduction

Chagas disease

Causal agent: Protozoan *Trypanosoma cruzi*

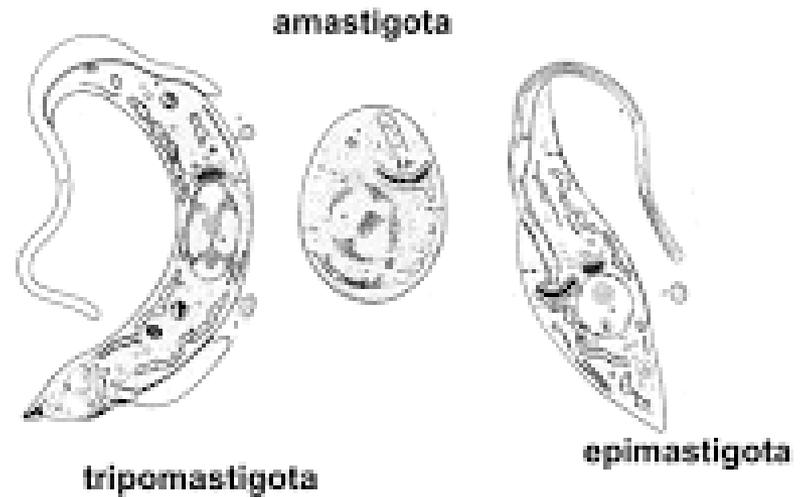
Morphology: Change through life cycle

Vector: *Triatoma infestans*



Disease stages:

- Acute:
 - Duration: 2 month after infection
 - most cases symptoms are absent
 - May appear skin lesion or a purplish swelling of the lids of one eye (Romania sign), fever, headache, enlarged lymph glands, pallor, muscle pain, difficulty in breathing, swelling, and abdominal or chest pain.
- Chronic:
 - Parasites are hidden mainly in the heart and digestive muscles
 - Cardiac, digestive and neurological disorders that can lead to sudden death



Morphological states

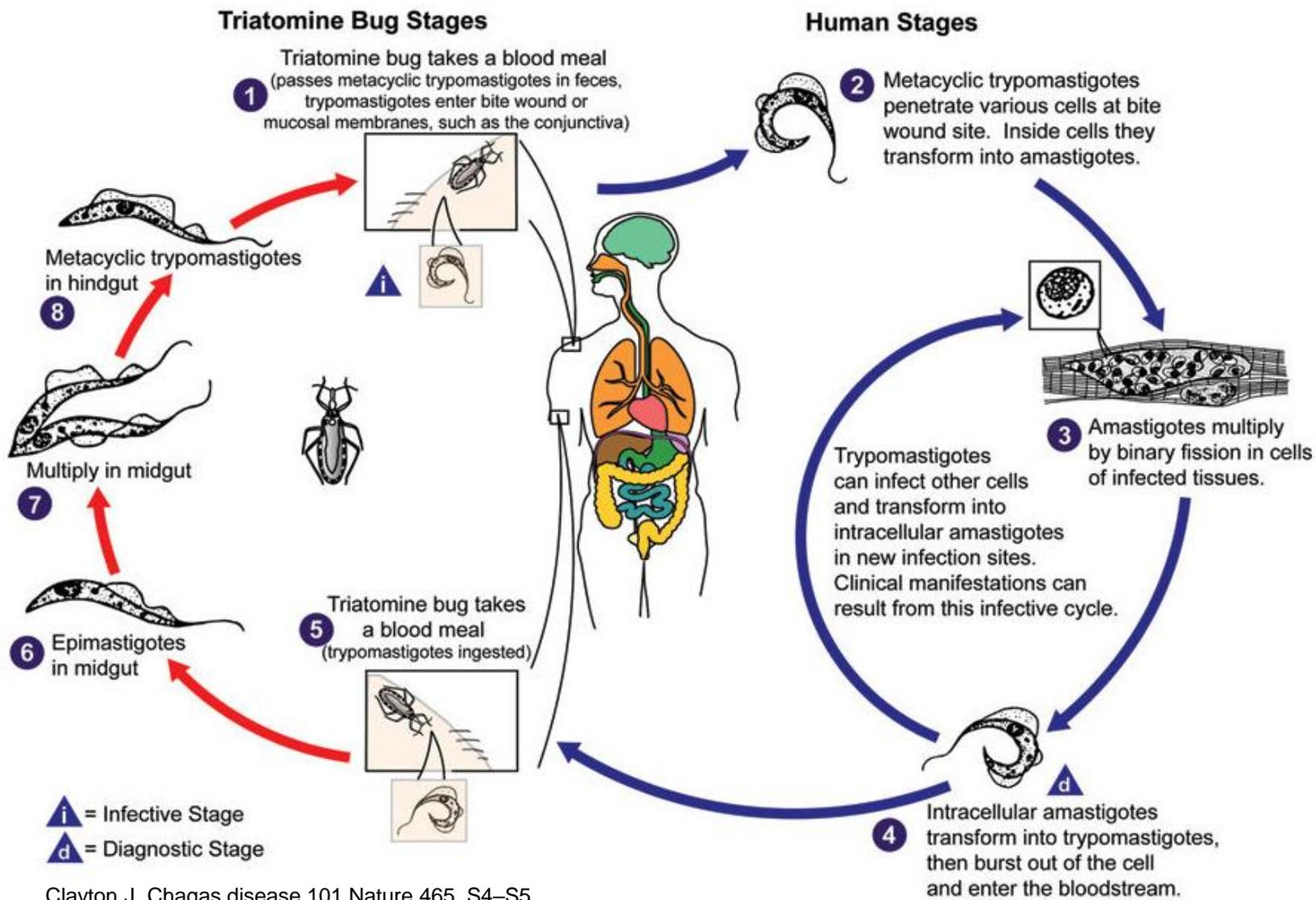


Romania sign



Introduction

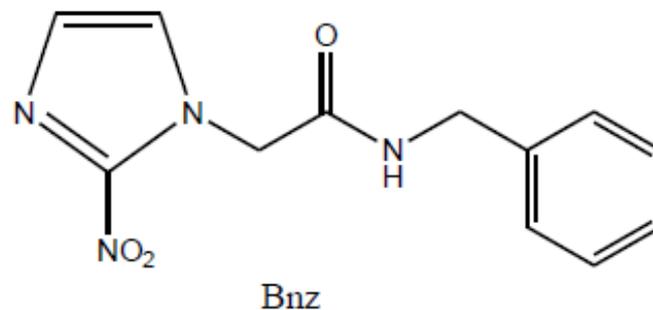
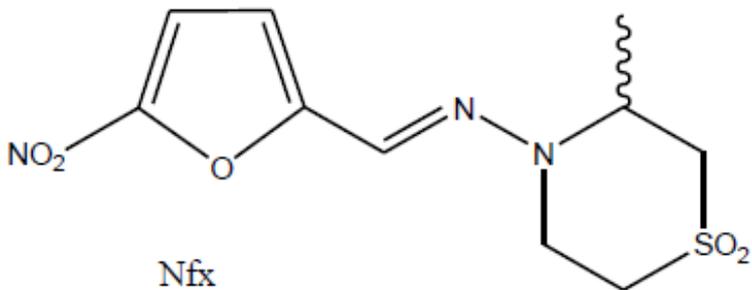
T-cruzi Vital cycle:



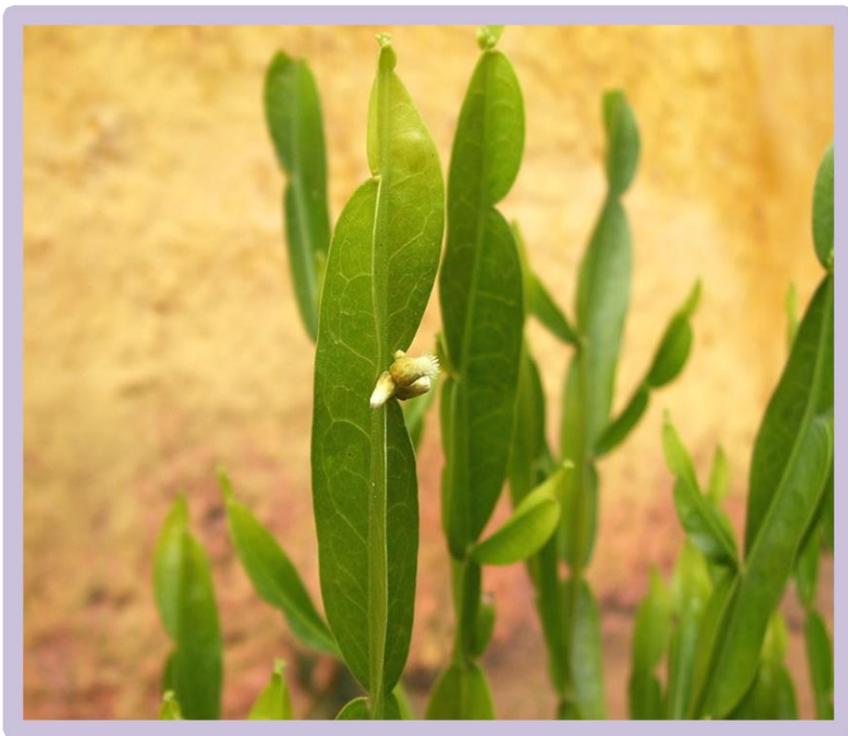
Introduction

Available treatments:

- Nifurtimox ,nitrofurfurylidene-amino (Nfx), Benznidazole, nitroimidazole acetamide (Bnz)
- Mechanism of action:
 - Formation of nitro-anion radical metabolite that reacts with nucleic acids of the parasite causing significant break down of DNA.
- Both are not completely effective against the disease.



Introduction



- Popular medicine recommends it to combat digestive and liver problems.

Carqueja

- Scientific name: *Baccharis trimera*
- Popular name: Carqueja
- Morphology: Bush up to 70 cm high, stems trialled, lacking leaves or with reduced leaves
- Geographic distribution: southern Brazil, Argentina, Paraguay and Uruguay.
- Flowering period: Late summer to early autumn.
- Some reported compounds: flavonoids such as santonine, absintine, quercetin, diterpenes: neoclerodane type.
- Reported properties: antioxidant, anti-inflammatory, **anti fungic**, bacteriostatic and bactericidal among other.

Nunes de Oliveira R. Garcia Rehder V. Marques S, et al. Schistosoma mansoni: In vitro schistosomicidal activity of essential oil of Baccharis trimera (less) DC . Experimental Parasitology 132 (2012) 135–143.



3rd International Electronic Conference
on Medicinal Chemistry
1-30 November 2017

sponsors:



pharmaceuticals

Introduction

Why Carqueja?

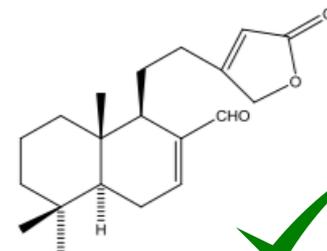
Antifungic effect related to tripanosomicide activity



FSC - Luis A. Funez

Relation between labdane containing aldehyde and biological activity

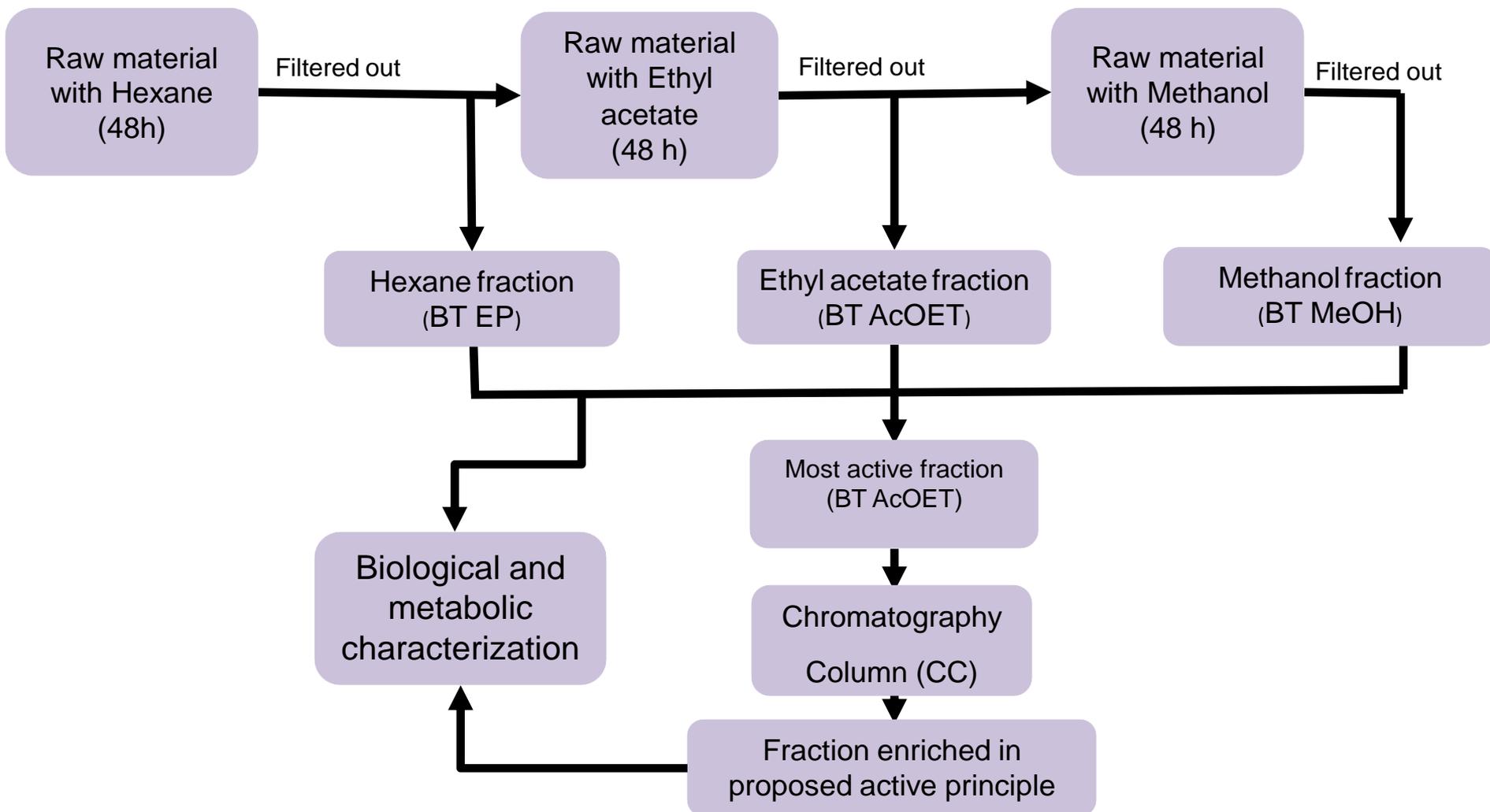
Lactone sesquiterpen present in many plants of *Asteraceae* with tripanosomicide activity



Metabolites labdane containing aldehyde previously isolated by the group in Matico (*Aristeguetia glutinosa*) are effective against *T. cruzi*



Materials and Methods



Materials and Methods

Antiproliferative activity assay:

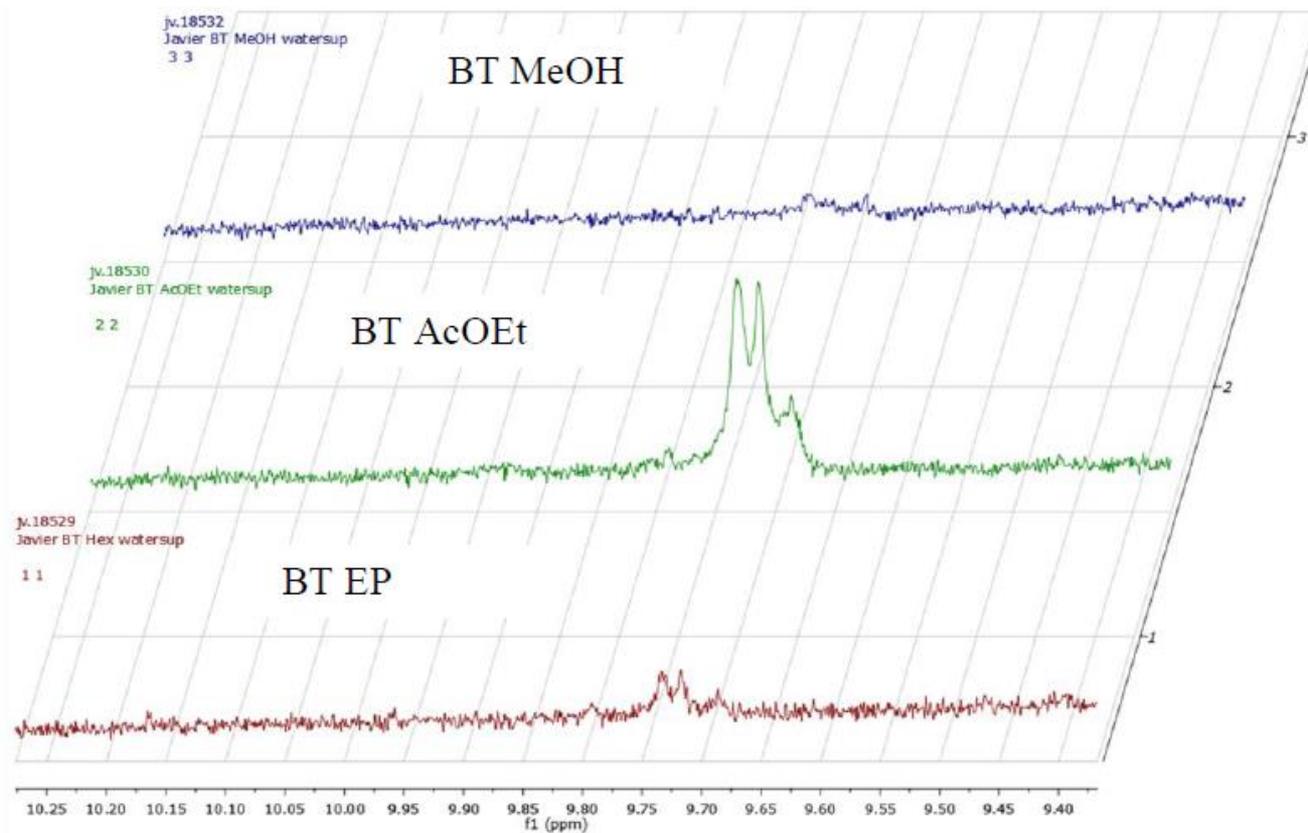
- Use cultures of *T. cruzi*, (epimastigote state) Tulahuen 2 strain, at 28 ° C (in exponential phase of growth)
- 0.6 mL / well is inoculated into a 24-well plate of a suspension of parasites at a concentration of 4 million cells / mL
- The parasites are incubated with the extracts at 28 ° C for 5 days.
- Measure of absorbance at 610 nm, (proportional to the number of cells) on days 0 and 5.
- The percent growth inhibition of the parasite is calculated according to:

$$\% = \{1 - [(A_p - A_{0p}) / (A_c - A_{0c})]\} \times 100$$

- **A_p**: Abs₆₁₀ day 5; **A_{0p}**: Abs₆₁₀ nm day 0; **A_c**: Abs₆₁₀ nm absence of compound day 5 (negative control); **A_{0c}**: Abs₆₁₀ nm absence of compound day 0
- The IC₅₀ is determined by plotting log (concentration) vs. % Inhibition of growth, adjusting the points to a Boltzmann Sigmoid curve.



Results and discussion



¹H-NMR spectra of *Baccharis trimera* samples corresponding to the three extracts amplified in the aldehyde zone.



Results and discussion

Table for integration of the region 9.76 - 9.70 ppm for the *Baccharis trimera* fractions

Fraction	Integration TMS	diterpenic aldehyde integration
BT EP	10	2,93
Bt Acoet	10	8,26
BT Meoh	10	2,24

Antiproliferative activity against epimastigote form of *T. cruzi*

Fraction	Yield (%)	IC ₅₀ (µg/mL)
BT EP	6	37.9 ± 2.1
BT Acoet	9	33.5 ± 1.6
BT Meoh	12	57.4 ± 2.4

- Ethyl acetate fraction presents greater biological activity followed by the hexanic fraction and finally the methanolic.



Results and discussion

Tracing of aldehyde compound in column chromatography of Ethyl acetate fraction by TLC



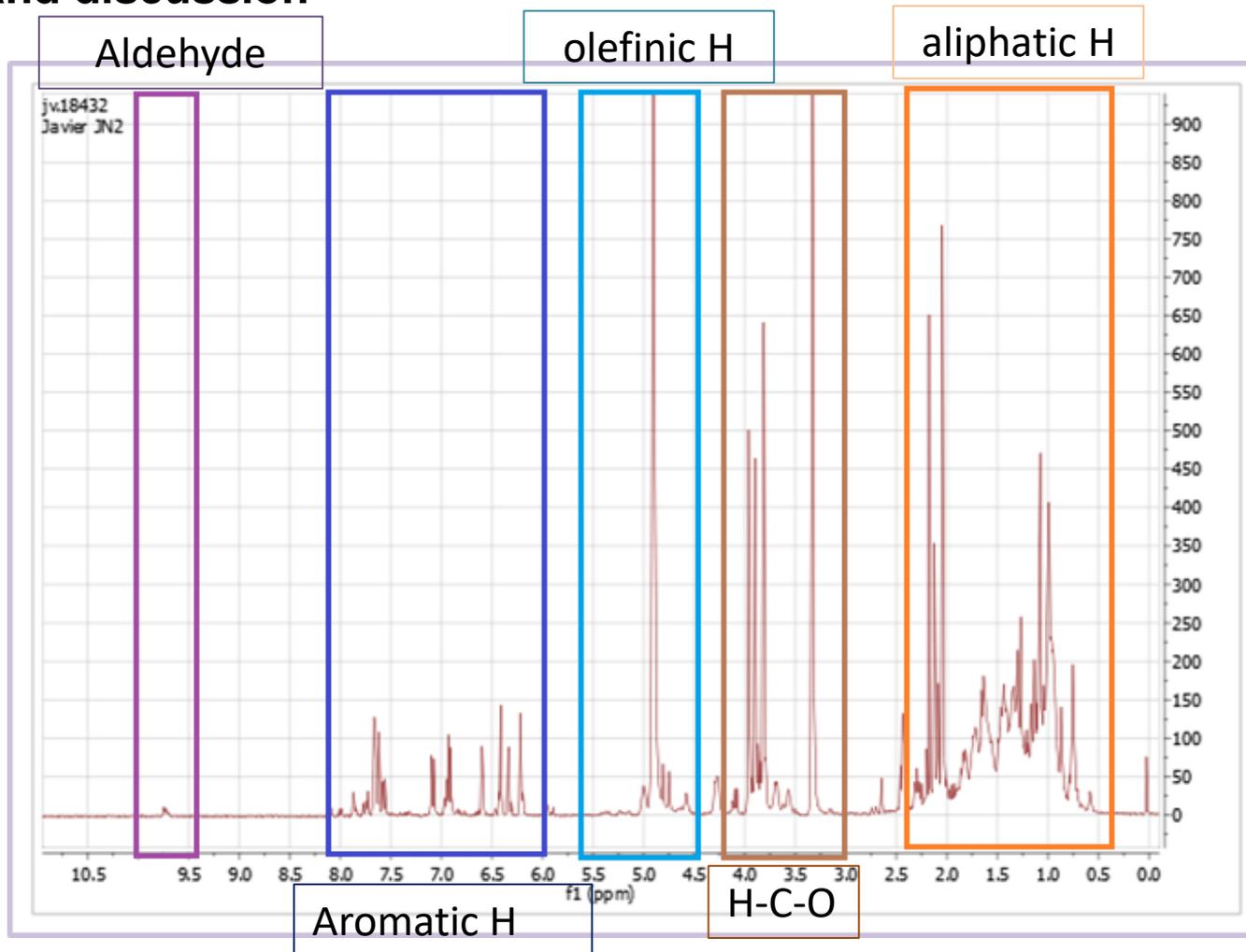
TLC of the fraction eluated with 1:1:1 (Hexane, Ethyl acetate, Chloroform)

- Mobile phase 1:1 (Ethyl acetate , Chloroform),
- Vanillin-sulfuric acid reagent pecific for terpenes (a)
- Brady reagent (specific for aldehyde or ketone groups) (b)

- **Yield : 6 %**



Results and discussion

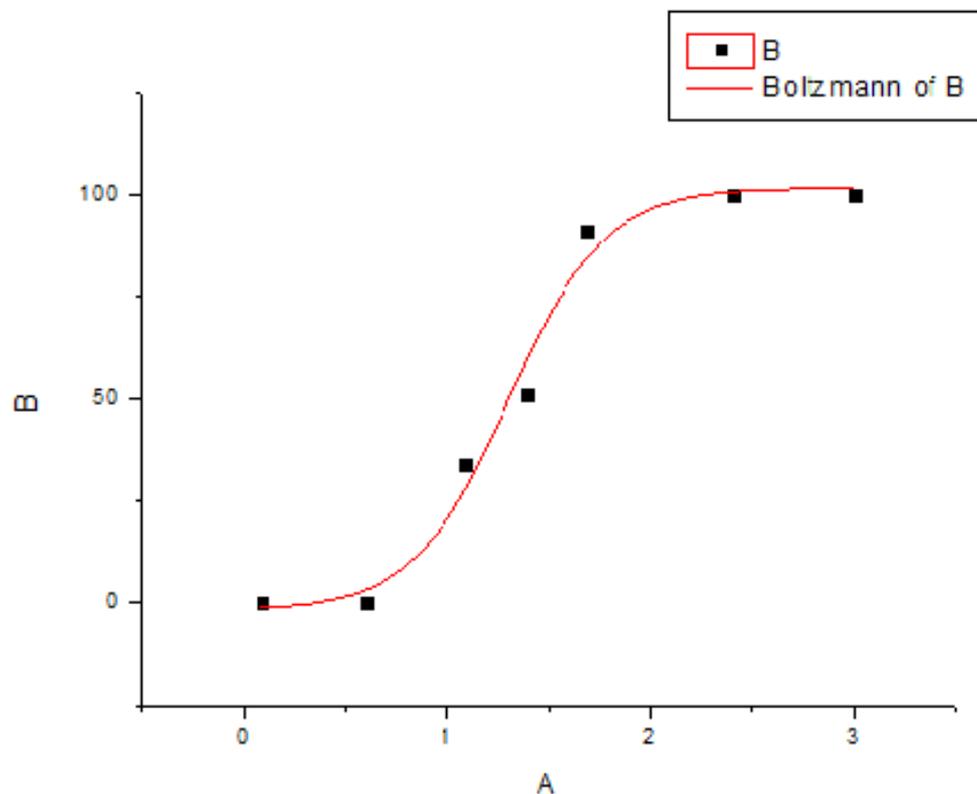


The presence of the aldehyde compound was confirmed by $^1\text{H-NMR}$ spectroscopy in the 1:1:1 fraction obtained by column chromatography of BT AcOEt



Results and discussion

Antiproliferative activity assay of purified fraction



Fraction	IC ₅₀ (ug/ml)
1:1:1	20,2 ± 7,0



Results and discussion

- In the initial fractionation carried out with the gradient of increasing polarity, it is observed that the ethyl acetate fraction presents greater biological activity, this coincides with what was expected, since this fraction is the one with the highest concentration of the active principle proposed by the metabolomic analysis. The hexanic fraction also presents an important activity, which may be due to the high presence of sesquiterpenic compounds and diterpenes. Even the methanolic fraction has considerable activity, this also shows that there are several with harmful compounds for the parasite in the plant, which present different polarity
- From the ^1H RMN spectra of the *Baccharis trimera* fractions obtained with first fractionation is seen the presence of two peaks in the region of aldehydes, whereby the diterpene may contain two aldehyde groups.
- Since the AcOEt fraction is the one with the highest concentration of the active ingredient and also the one with the highest biological activity, it was decided to purify it from this fraction, the mobile phase used to eluate the metabolite is 1:1:1 (Hexane, Ethyl acetate, Chloroform) The thin layer chromatography shows that with Vanillin reagent the fraction 111 has spots at $r_f = 0.52$ and $r_f = 0.66$, the first one reveals violet in Vanillin and orange in Brady, corresponding to a terpene with an aldehyde in its structure, the second reveals yellow in Vanillin which implies the presence of flavonoids in the area and yellow in Brady, due to ketone groups characteristic of these compounds.
- The spectrum obtained with de ^1H - RMN analysis allow to confirm the presence of the aldehyde in the fraction but not to elucidate in detail the structure. Also we can see protons corresponding to aromatic rings due to flavonoids putting on evidence the presence of other compounds in the fraction thus more purification steps will be necessary.



Conclusions

- *Bacharis trimera*, is an important source of anti- *trypanosoma cruzi* agents.
- Polarity gradient fractionation demonstrates that there are several compounds with trypanosomicidal activity.
- The fraction enriched in the aldehyde presents a relevant activity so we can infer that plays an important role in the observed growth inhibition of the entire extract.
- Further purification steps are necessary to confirm the structure and activity of diterpene it self versus *T. cruzi*.



Acknowledgments



GRUPO
**QUIMICA
medicinal**



3rd International Electronic Conference
on Medicinal Chemistry
1-30 November 2017

sponsors:



pharmaceuticals