Bioguided Fractionation of Modified Plant Extract: An Efficient Approach for Discovering Bioactive Compounds and Identifying Biological Targets

Tonino G. Adessi [a]; Viviana E. Nicotra [b]; Manuela E. García.[b]

[a] Instituto de Química Rosario (IQUIR-CONICET), Rosario, S2000, Argentina; [b] Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Córdoba, 5000, Argentina.

adessicofre@iquir-conicet.gov.ar

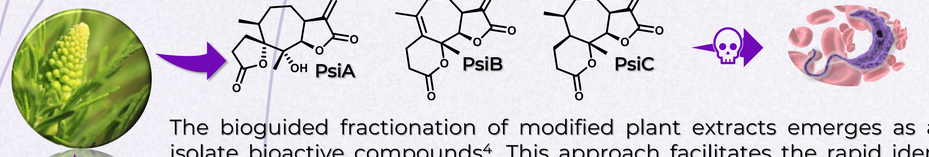
Abstract

This study presents a methodology for the bioactivity-guided fractionation of chemically modified Ambrosia tenuifolia extracts, employing mild derivatization conditions at room temperature. New trypanocidal compounds, which are less toxic than the starting materials, are obtained in a single reaction step. Additionally, bioinformatics tools were used to identify potential biological targets for Trypanosoma cruzi, reducing the need for extensive biological testing. This strategy optimizes resource utilization in both organic and biological laboratories by simulating interactions and discarding less promising compounds, thus accelerating the drug discovery process.

Introduction

Natural products (NPs) are unparalleled sources of lead structures for drug discovery.[1]

The native Argentinean plant *Ambrosia tenuifolia* is rich in sesquiterpene lactones, such as Psilostachyins (Psi)[2], known for their trypanocidal potential[3].



The bioguided fractionation of modified plant extracts emerges as an efficient strategy to isolate bioactive compounds[4]. This approach facilitates the rapid identification of promising molecules by focusing on the most active fractions, thereby streamlining the discovery process and optimizing resource use.

Objectives

To establish a bioguided fractionation methodology for modified plant extracts that facilitates the rapid identification of trypanocidal derivatives of natural products, minimizing the need for exhaustive purification of all compounds and optimizing solvent use by reducing the number of derivatization assays required.

Apply bioinformatics tools to the identified bioactive chemical entities to propose biological targets associated with Chagas disease, allowing experimental efforts to be directed towards specific pathways and reducing the need for extensive testing of all known targets.

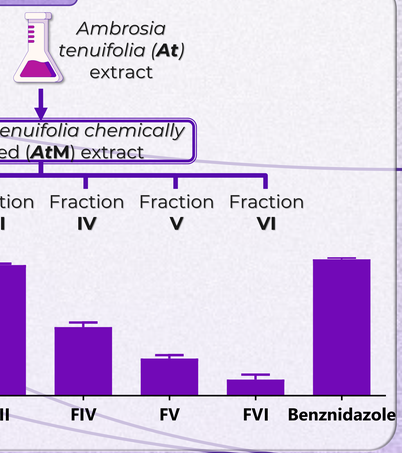
Bioguided Fractionation

Chemical modification:
Plant extract in dichloromethane (DCM), 0.02 g/mL *m*-chloroperoxybenzoic acid (mCPBA), 0.088 M 24 hs at Room temperature

Fractionation
The fractionation of the modified extract was carried out using Flash chromatography, employing ethyl acetate and hexane as solvents, with the minimum necessary volumes

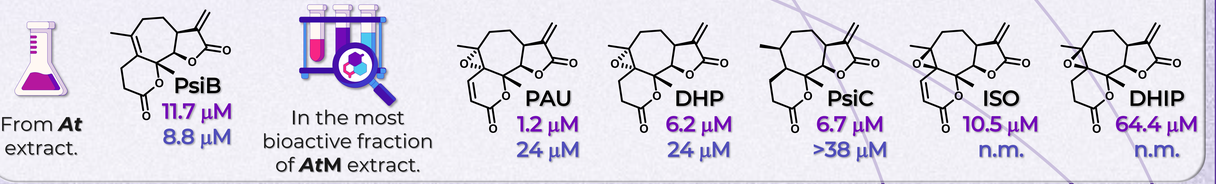
Bioactivity measurement:
Percentage of *T. cruzi* trypomastigote inhibition at a concentration of 5 µg/mL, and the results were compared with a reference drug at the same concentration.

Benznidazole: IC_{50}: 4.2 µM



Bioactive molecules

After the purification of only **Fraction III**, the trypanocidal activity (IC_{50}) of its components and the PsiB compound, identified as the major NP in the ***At*** extract, was measured. Additionally, cytotoxicity (CC_{50}) was evaluated using the RAW 264.7 macrophage cell line.



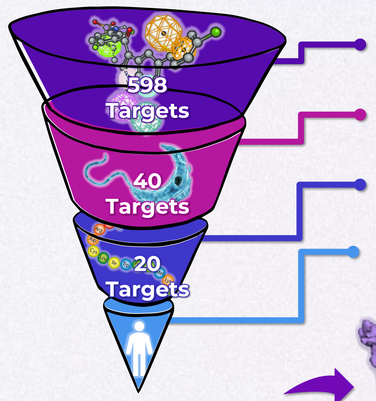
Potential biological targets

With the structure of six trypanocidal molecules (PsiB, PsiC, PAU, DHP, ISO, and DHIP), a bioinformatic analysis was performed to propose biological targets in the metabolism of *Trypanosoma cruzi*.

The most (**PAU**) and least trypanocidal compounds (**DHIP**) were submitted to a reverse pharmacophore mapping server

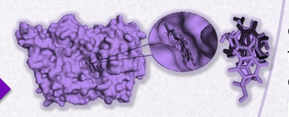
PharmMapper

This server contains a database of pharmacophore models for protein active sites.[5]



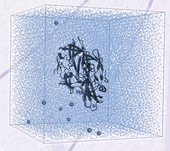
4 Potential Targets:

- Dihydroorotate dehydrogenase A
- Glucose-6-phosphate isomerase
- Triosephosphate isomerase
- Trypanothione reductase (**TR**)



The six trypanocidal compounds showed favorable molecular docking poses with the potential targets.

The three most trypanocidal compounds (**PAU**, **DHP**, and **PsiC**) formed stable complexes with TR in molecular dynamics, and favorable binding free energies were calculated.



Conclusions

- The methodology used in this study represents an advance over traditional pure natural product derivatization, allowing efficient and rapid identification of bioactive compounds by focusing on the most active fractions. This approach yielded a compound (**PAU**) more potent than the reference drug and with low toxicity. Although a versatile and accessible oxidant was used, further work is needed to develop more environmentally friendly reaction methods.
- In addition, bioinformatics studies of the bioactive compounds identified four potential molecular targets in *T. cruzi*. This targeted approach streamlines biochemical studies by suggesting a theoretical mode of action, reducing reagent consumption and the need for extensive biological assays, thereby optimizing laboratory resources.

Acknowledgments

CONICET · AGENCIA Nacional de Promoción Científica y Tecnológica · FCQ Facultad de Ciencias Químicas · UNC Universidad Nacional de Córdoba · SECyT Secretaría Ciencia y Tecnología · Office of Naval Research Global Science & Technology

References

1. D. Newman, G. Cragg J. Nat. Prod. 2020, 83, 770-803.;
2. Silva, G. L., et al. (1992). Phytochemistry, 31(3), 859-861.
3. Sülsen, V. P., & Martino, V. S. (2018). Sesquiterpene lactones
4. Ramallo, I. A., et al. (2018). ACS combinatorial science, 20(4), 220-228.
5. Wang, X., Shen, Y., et al. (2017) Nucleic acids research, 45(W1), W356-W360.
6. Shanmugasundram, A., et al. (2023). PLOS Neglected Tropical Diseases, 17(1), e0011058.
7. Ogata, H., et al. (1998). Biosystems, 47(1-2), 119-128.
8. Madeira, F., et al. (2024). Nucleic Acids Research, gkae241.
9. UniProt Consortium. (2019). Nucleic acids research, 47(D1), D506-D515.