ResNet NPND

10th Anniversary
2011 - 2021

Anniversary Online Symposium

May 07, 2021, 14:00 – 21:00 MEST, online via Zoom

Report

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10th Anniversary
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Organizer's remarks

The 10th anniversary symposium of ResNet NPND was held on May 07, 2021 as an online conference via Zoom. Almost exactly ten years after the founding conference, numerous members of the network and their affiliates came together in a truly global reunion and shared almost seven hours of science! ResNet NPND Members from all over the world, i.e. from Africa (Kenya, Nigeria, Sudan, Tunisia), the Americas (Argentina, Brazil, Colombia, Uruguay, USA), Europe (Germany, France, Switzerland, UK) and Asia (South Korea) attended the meeting. Representatives of the various research groups reported in 20 excellent lectures on their latest findings in the fight against Neglected Tropical Diseases with Natural Products. Most of these presentations were held by young scientists who currently work as doctoral students or post doc scientists in the various groups represented at the meeting and it was thus a big pleasure to see that the network may look forward into a bright future.

As the organizer, I would like to extend very cordial thanks to Prof. Dr. Stephan Ludwig, Former Vice-Rector for Research and present Head of the Research Council of WWU Münster and to Prof. Dr. Joachim Jose, Dean of the Faculty of Chemistry and Pharmacy of WWU, for their thoughtful and very encouraging words expressed in their greeting addresses during the opening of the conference.

Most of all, big thanks are due to all who actively contributed to the scientific program of this successful meeting, not only to the numerous speakers who shared their exciting results, but also to those who contributed with their constructive questions and points of discussion and to those who just attended and thus provided a wonderful audience.

This document, with photographs of the various speakers and abstracts of their presentations, gives a short overview on the breadth of a major part of the network's ongoing research activities. It may serve as a memory, maybe, to look back again after ten more years, but currently, it is certainly a good starting point to look forward into this coming next decade of the network!

Thomas J. Schmidt

Münster, May 10, 2021

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Program

(Time is Middle European Summer Time – Amsterdam, Münster, Berlin)

14:00 – 14:30	***Welcome and greeting addresses*** Prof. Dr. Stephan Ludwig – Former Vice-Rector for Research, acting Head of the Research Council of WWU Münster Prof. Dr. Joachim Jose – Dean of the Faculty of Chemistry and Pharmacy, WWU Münster Prof. Dr. Thomas J. Schmidt, WWU Münster, Coordinator of ResNet NPND
14:30 – 15:15	***Anniversary highlight lecture*** Parasites that turned the wheel of history Prof. Dr Pascal Mäser, Swiss Tropical and Public Health Institute, Basel, Switzerland:
15:15 – 15:30	***ResNet NPND: Looking back, we look forward*** Prof. Dr. Thomas J. Schmidt, Münster, Germany
	Short Lectures (only presenting authors mentioned here; for full authorship see abstracts below)
15:30 – 15:40	**Patricia Sartorelli, São Paulo, Brazil:** Molecular network for accessing polyketide derivatives from *Phomopsis* sp., an endophytic fungus of *Casearia arborea* (Salicaceae) and evaluation of antiparasitic activity
15:40 – 15:50	**Jimena Borgo, Buenos Aires, Argentina:** Anti-*Trypanosoma cruzi* activity of a sesquiterpene lactone isolated from *Stevia satureiifolia* var. *satureiifolia* –
15:50 – 16:00	**Thais Alves da Costa Silva, São Paulo, Brazil:** Diterpenes isolated from *Baccharis sphenophylla* (Asteraceae) rapidly kill *Trypanosoma cruzi* by alteration in acidocalcisomes organelles and in ATP synthesis
16:00 – 16:10	**Simone Dos S. Grecco, São Paulo, Brazil:** Investigation of anti-parasitic effect of neolignans isolated from *Nectandra leucantha* (Lauraceae) using countercurrent chromatography
16:10 – 16:20	**Erica V. de Castro Levatti, São Paulo Brazil:** Mitochondrial Imbalance Caused by Semi-Synthetic Derivatives of Licarin A in *Leishmania (L.) infantum*
16:20 – 16:35	***Break and discussion***
16:35 – 16:45	**Vitor Lourenzon, Ribeirao Preto, Brazil:** Biosynthetic pathway of cyphomycin, an antileishmanial polyketide produced by ant-associated *Streptomyces*
16:45 – 16:55	**Ghozlene Mekhloufi, Paris, France:** In vitro antileishmanial properties of essential oil from Tunisian *Citrus limon*
16:55 – 17:05	**Franziska M. Jürgens, Münster, Germany:** Investigation of antileishmanial constituents in *Arnica* flowers
17:05 – 17:15	**Chonny A. Herrera Acevedo, João Pessoa, Brazil & Cajica, Colombia:** Brazil – Colombia cooperation in cheminformatics studies against leishmaniasis: looking for terpenoid-based hits
17:15 – 17:25	**Katharina Possart, Münster, Germany:** Rational search for natural inhibitors of the *Trypanosoma brucei* pteridine metabolism
17:25 – 17:40	***Break and discussion***
17:40 – 17:50	**Florencia Sardi, Montevideo, Uruguay:** Insights into the mechanism of action of natural sesquiterpene lactones against pathogenic trypanosomatids
17:50 – 18:00	**Lara U. Szabó, Münster, Germany:** Antitrypanosomal Alkaloids of *Buxus sempervirens* L.
18:00 – 18:10	**Kamila T. Yuyama, Ribeirao Preto, Brazil & Münster, Germany:** Discovering the potential of the secondary metabolites from *Malouetia tamaquarina* (Apocynaceae) and its endophytes against neglected tropical diseases

18:10 – 18:20	**Abdelhalim Babiker M. Mahmoud, Khartoum/Omdurman, Sudan:** HPLC-Based Activity profiling of Sudanese Medicinal Plants for Natural Compounds against Tropical Parasitic Diseases
18:20 – 18:30	**John O. Igoli, Markurdi, Nigeria:** Propolis against kinetoplastid parasites
18:30 – 18:45	***Break and discussion***
18:45 – 18:55	**Taylor Diaz-Herrera, Cacjicá, Colombia:** Anti-dengue Activity of *Phyllanthus urinaria* against four DENV serotypes
18:55 – 19:10	**Clara Albani, Mar del Plata, Argentina:** In vitro and In vivo anthelmintic activity of *Stevia multiaristata* extract on *Echinococcus granulosus*.
19:10 – 19:20	**Shereen O. Abd Algaffar, Khartoum/Omdurman, Sudan:** Drug Discovery of Natural Bioactive Molecules against *Madurella mycetomatis*- One of the Most Neglected Diseases
19:20 – 19:35	***Break and discussion***
19:35 – 20:00	***Network highlight lecture:*** **Sami A. Khalid, Khartoum/Omdurman, Sudan:** Mycetoma Drug Discovery Research Platform Conslidates the Concept of Networking
20:00 – open end	***Online – Celebration - Chatrooms***

ResNet NPND
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Scientific Contributions

(in the order of their appearance in the symposium program)

Anniversary highlight lecture

Parasites that turned the wheel of history

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The COVID-19 pandemic has shown that modern societies are still vulnerable to infectious diseases. But how much more was this the case before people knew about the causative agents of disease and their routes of transmission. It was not until the dawn of the 20th century, after the golden age of microbiology had been initiated by Pasteur and Koch, that effective measures were taken to combat such scourges as malaria, yellow fever, or helminth infections. Earlier, the chosen countermeasures were often useless or even detrimental. Imagine that the French doctors in the West Indies were treating yellow fever and malaria by bloodletting, or that during the first attempt to build the Panama Canal the workers' beds were mounted in cans of water to ward off insects, unwittingly providing the perfect breeding ground for *Anopheles* mosquitoes. No wonder that such practices invited disaster. Using examples from the mysterious St. Gotthard disease in Switzerland to the failed attempt of Napoleon to build an empire in the New World, I shall try to illustrate how pathogens have been influencing human history and shaping the fates of whole nations.

Molecular network for accessing antiparasitic compounds from *Phomopsis* sp., an endophytic fungus of *Casearia arborea* (Salicaceae)

Augusto L. dos Santos[1], Maiara Amaral[2], Maiara M. Romanelli[2], Erica C. Levatti[2], André G. Tempone[2] and Patricia Sartorelli[1]*

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In the last three decades endophytes have shown to be a promising source for the discovery of new antiparasitic prototypes [1]. Considering the large number of metabolites from endophytes that can be prospected, fractionation and isolation of metabolites can be an arduous task. Therefore, tools for rapid identification of molecules *in-situ* have emerged as an important tool in helping to easily and quickly identify molecules that may have antiparasitic activity, as a device that makes it possible to identify known compounds and prospect new ones [2]. Therefore, dereplication platforms for rapid annotation of metabolites, as well as creation of molecular networks, as GNPS (*Global Natural Products Social Molecular Networking*) has emerged as a global, interactive, and *online* platform, in which *tandem* mass spectra (MS^2) obtained from high resolution mass spectrometers can be analyzed and grouped, resulting in the creation of molecular networks, as well as the annotation of molecules present in the database [3]. In this way EtOAc crude extract obtained from the endophytic fungus *Phomopsis* sp. isolated from leaves of *C. arborea* was dereplicated using GNPS platform, based on the molecular networking obtained from UHPLC-HR-ESI-MS/MS spectral data. The used approach pointed towards different polyketide spectral families produced by the strain. Therefore, the extract was fractionated and seven derivatives were isolated. The chemical structures were elucidated through 1D

and 2D NMR and HR-ESI-MS data analysis. Additionally, the activity of these compounds was evaluated against trypomastigote and amastigote forms of *T. cruzi*. The obtained results indicated a promising compound named cytosporone B against trypomastigotes and amasitogtes of *Trypanosoma cruzi*, with IC_{50} of 36,5 and 9,1 μg/mL respectively and no cytotoxicity against NCTC at concentration of 200 μg/mL.

[1] Kaul, S.; Gupta, S.; Ahmed, M.; Dhar, M.K. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochem Rev* **2012**, 11, 487. doi: 10.1007/s11101-012-9260-6

[2] Hubert, J.; Nuzillard, J.M.; Renault., J.H. Dereplication strategies in natural product research: How many tools and methodologies behind the same concept? *Phytochem Rev* **2017**, 16, 55. doi: 10.1007/s11101-015-9448-7

[3] Aron, A.T.; Gentry, E.C.; McPhail, K.L.; *et al*., Reproducible molecular Network of untargeted mass spectrometry data using GNPS. *Nat Protoc* **2020**, 15, 1954. doi: 10.1038/s41596-020-0317-5.

A trypanocidal sesquiterpene lactone isolated from *Stevia satureiifolia* var. *satureiifolia*

Jimena Borgo[1,2]*, Augusto Bivona[3], M. Florencia Beer[1,2], Natacha Cerny[3], Marlene Cabral[2], Natalia Hernandez[2], Andrés Sanchez Alberti[3], Emilio Malchiodi[3], Cesar Catalán[4], Florencia Martini[1,5] and Valeria P. Sülsen[1,2]
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American trypanosomiasis or Chagas disease is an endemic parasitosis caused by the protozoan *Trypanosoma cruzi* that affects 6 to 7 million people worldwide, mostly in Latin America and is responsible for more than 10000 deaths per year [1]. Benznidazole and nifurtimox are the only available drugs for its treatment. These antiparasitic drugs were developed half a century ago and present variable efficacy and fairly common side effects. Consequently, new therapeutic agents to treat this disease are urgently needed.

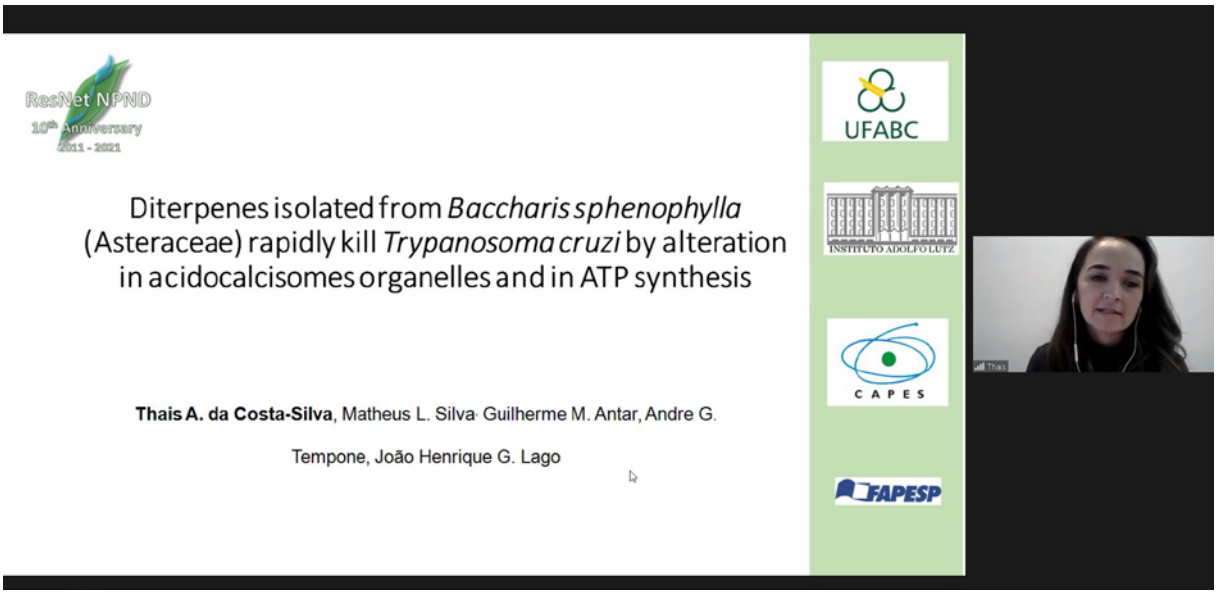
As a result of our ongoing research on antiparasitic compounds from *Stevia* species, we have reported the trypanocidal activity of the organic extract of *S. satureiifolia* var. *satureiifolia* (Asteraceae) and the isolation of the active flavonoids eupatorin and 5-desmethylsinensetin [2]. Based on these results, we have continued the study of this extract in the search of other bioactive compounds.

Dried aerial parts of *S. satureiifolia* var. *satureiifolia* were extracted by maceration with dichloromethane. Fractionation of this extract was performed by column chromatography using Silicagel and a gradient of dichloromethane:EtOAc. Seven fractions (F_A to F_G) were obtained. The

purification of fraction D (F_D), eluted with dichloromethane:EtOAc (2:1), afforded a precipitate (compound A). The purity of the compound was assessed by chromatographic techniques and the structure elucidation was performed by spectroscopic methods. The compound was identified as a germacranolide type sesquiterpene lactone. The *in vitro* activity of this sesquiterpene lactone against *T. cruzi* epimastigotes and amastigotes was tested, resulting in IC_{50} values of 1.75 and 6.33 µg/ml, respectively. Evaluation of the activity against trypomastigote forms of the parasite, which constitutes the predominant form in the acute phase of the disease, is currently in progress.

[1] World Health Organization (WHO), 2021. Fact Sheet Chagas disease (American trypanosomiasis). 1 April 2021. Available at https://www.who.int/en/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)

[2] Beer M.F. et al., 2016. *Pharm Biol*. 54(10):2188-95.



Diterpenes isolated from *Baccharis sphenophylla* (Asteraceae) rapidly kill *Trypanosoma cruzi* by alteration in acidocalcisomes organelles and in ATP synthesis

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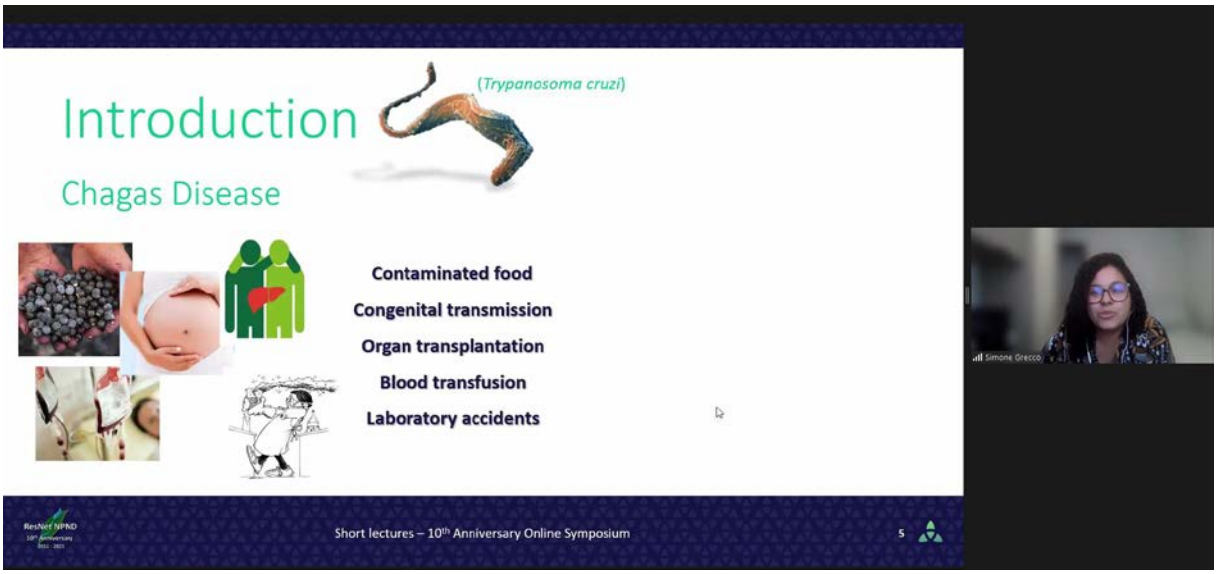
Distributed primarily in the tropical areas of South America, the genus *Baccharis* is represented by more than 500 species and was phytochemically composed by diterpenes and flavonoids [1]. In the present study, the bioactivity-guided fractionation of n-hexane extract from *B. sphenophylla* against *Trypanosoma cruzi* afforded three related diterpenoids: *ent*-kaurenoic acid (**1**), grandifloric acid (**2**), and 15β-tigloyl-*ent*-kaurenoic acid (**3**). The structures of isolated compounds were defined by spectroscopic analysis. Antitrypanosomal activity of **1 – 3** was performed against cell-derived trypomastigotes while cytotoxicity was evaluated against NCTC cells [3]. As results, compound **3** displayed a moderate toxicity (CC_{50} = 83.5 µM) while compounds **1** and **2** were non-toxic (CC_{50} > 200 µM). Regarding the antitrypanosomal activity, compounds **1** and **3** were effective against trypomastigotes with IC_{50} 10.6 and 2.4 µM, respectively, indicating higher potential in comparison to positive control benznidazol (IC_{50} = 18.7 µM). Thereafter, their mechanism of action against the parasite was studied [2]. Initially, was observed that both compounds have no action in the plasma membrane permeability. Nevertheless, compound **1** induced a disturbance in the plasma membrane electric potential (ΔΨp) while compound **3** also induced alteration in this parameter, but not statistically significant. Compound **1** also induced mitochondrial depolarization (ΔΨm) and alteration in the acidocalcisomes, while compound **3** showed alteration exclusively in the acidocalcisomes. The

single mitochondria and acidocalcisomes are Ca^{2+} compartments and constitute important chemotherapeutic target in trypanosomatids. Both organelles are a source of ions, and the alteration in the acidocalcisomes could be related with these molecules [3]. Finally, an increase in the ATP levels was observed after treatment with compounds **1** and **3**. In conclusion, compounds **1** and **2** displayed an apparent action in ATP synthesis and acidocalcisomes suggesting that these natural compounds could be used as scaffolds to development of new drugs to treatment of Chagas Disease.

[1] Ueno, A.K.; Barcellos, A.F.; Grecco, S.S.; Sartorelli, P.; Guadagnin, R.C.; Romoff, P.; Ferreira, M.J.P.; Tcacenco, C.M.; Lago, J.H.G. Sesquiterpenes, diterpenes, alkenyl *p*-coumarates, and flavonoid from the aerial parts of *Baccharis retusa* (Asteraceae). *Biochem Syst Ecol* **2018**, 78, 29-32. doi: 10.1016/j.bse.2018.03.013

[2] Oliveira, E.A.; Brito, I.A.; Lima, M.L.; Romanelli, M.; Moreira-Filho, J.T.; Neves, B.J.; Andrade, C.H.; Sartorelli, P.; Tempone, A.G.; Costa-Silva, T.A.; Lago, J.H.G. Antitrypanosomal activity of acetogenins isolated from the seeds of *Porcelia macrocarpa* is associated with alterations in both plasma membrane electric potential and mitochondrial membrane potential. *J Nat Prod* **2019**, 82, 1177-1182. doi: 10.1021/acs.jnatprod.8b00890

[3] Huang, G.; Ulrich, P.N.; Storey, M.; Johnson, D.; Tischer, J.; Tovar, J.A.; Moreno, S.N.; Orlando, R.; Docampo, R. Proteomic analysis of the acidocalcisome, an organelle conserved from bacteria to human cells. *PLOS Pathog* **2014**, 12, e1004555. doi: 10.1371/journal.ppat.1004555



Investigation of anti-parasitic effect of neolignans isolated from *Nectandra leucantha* (Lauraceae) using countercurrent chromatography

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In our studies, the *n*-hexane extract from leaves of *Nectandra leucantha* (Lauraceae) displayed *in-vitro* activity against amastigote forms of *Trypanosoma cruzi* and *Leishmania infantum.* The bioactive secondary metabolite principles were isolated by means of all-liquid countercurrent chromatography (*high-performance*, and *spiral-coil*-CCC), monitored by *off-line* electrospray mass-spectrometry injection profiles [1]. Using this approach, five related neolignans were obtained: dehydrodieugenol (**1**) dehydrodieugenol B (**2**), 1-(8-propenyl)-3-[3'-methoxy-1'-(8-propenyl)-phenoxy]-4,5-dimethoxybenzene (**3**), 1-[(7*S*)-hydroxy-8-propenyl]-3-[3'-methoxy-1'-(8'-propenyl)-phenoxy]-4-hydroxy-5-methoxybenzene (**4**), and 1-[(7*S*)-hydroxy-8-propenyl]-3-[3'-methoxy-1'-(8'-propenyl)-phenoxy]-4,5-dimethoxybenzene (**5**) which were identified by analysis of NMR and MS data [2,3]. These compounds were tested *in vitro* against etiological agents from Chagas disease (*T. cruzi*) and visceral leishmaniasis (*L. infantum*) as well as for mammalian cytotoxicity (NCTC cells) [2-5]. Compounds **1** and **5** displayed higher activity against trypomastigote (EC_{50} of 11.5 and 39.2 μM) and amastigote (EC_{50} of 15.1 and 15.2 μM) forms of *T. cruzi*. Except to compound **1** (CC_{50} 58.2 μM) all isolated neolignans exhibited reduced toxicity against NCTC cells (CC_{50} > 200 μM). The investigation of the mechanism of action demonstrated that, after a short-term incubation, the fluidity and integrity

of the plasma membrane was completely altered, suggesting it as a primary target for compound **1** in *T. cruzi*. Additionally, neolignan **5** caused substantial alteration of the plasma membrane permeability, together with mitochondrial dysfunctions in trypomastigote forms. Neolignans **3** and **5** showed also potential against amastigote forms of *L. (L.) infantum* (EC_{50} values of 57.9 and 67.7 µM) while compound **1**, **2** and **4** were inactive. As these neolignans are chemically related, it may be suggested that the presence of the methoxyl group at C-4 constitutes an important structural aspect to increase antileishmanial potential against amastigote forms. Considering the promising chemical and biological properties of the isolated neolignans, these compounds could be used as starting points to develop new lead compounds for Chagas disease and leishmaniasis.

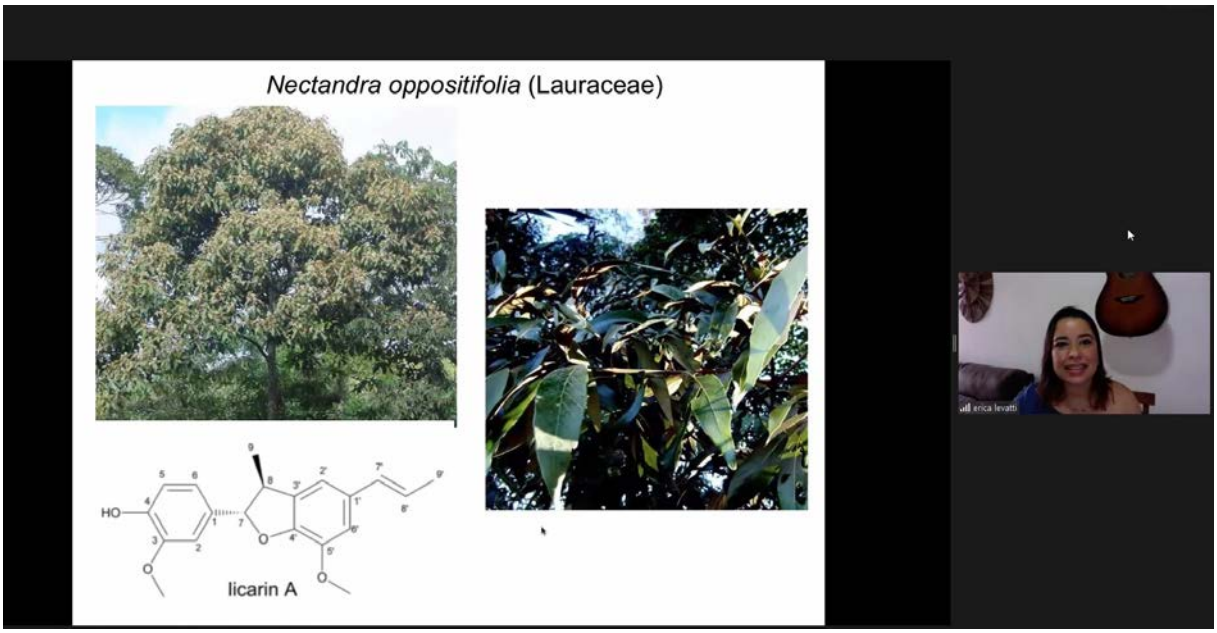
[1] Grecco, S.S.; Letsyo, E.; Tempone, A.G.; Lago, J.H.G.; Jerz, G.. Electrospray mass-spectrometry guided target isolation of neolignans from *Nectandra leucantha* (Lauraceae) by high performance-and spiral-coil countercurrent chromatography. *Journal of Chromatography A* **2019**, 1608, 460422.

[2] Grecco, S.S.; Costa-Silva T.A.; Jerz, G.; de Sousa, F.S; Alves Conserva, G.A.; Mesquita, J.T.; Galuppo, M.K.; Tempone, A.G.; Neves, B.J.; Andrade, C.H.; Cunha, R.L.O.R.; Uemi, M.; Sartorelli, P.; Lago, J.H.G. Antitrypanosomal activity and evaluation of the mechanism of action of dehydrodieugenol isolated from *Nectandra leucantha* (Lauraceae) and its methylated derivative against *Trypanosoma cruzi*. *Phytomedicine* **2017**, 24, 62–67.

[3] Grecco, S.S.; Costa-Silva,T.A.; Jerz, G.; de Sousa, F.S.; Londero, V.S.; Galuppo, M.K.; Lima , M.L.; Neves, B.J.; Andrade, C.H.; Tempone, A.G.; Lago, J.H.G. Neolignans from leaves of *Nectandra leucantha* (Lauraceae) display in vitro antitrypanosomal activity via plasma membrane and mitochondrial damages. *Chemico-Biological Interactions* **2017**, 277, 55-61.

[4] Grecco, S.S.; Jerz, G.; Lago, J.H.G.; Jones, P.G. Crystal structure of Dehydrodieugenol B methyl ether, a neolignan from *Nectandra leucantha* Nees and Mart. (Lauraceae). *Acta Crystallographica,* **2018**, E74, 518–521.

[5] Grecco, S.S.; Costa-Silva, T.; Sousa, F.S.; Cargnelutti, S.B.; Umehara, E.; Mendonça, P.S.; Tempone, A.G.; Lago, J.H.G. Neolignans isolated from twigs of *Nectandra leucantha* Ness & Mart (Lauraceae) displayed *in vitro* antileishmanial activity. *Journal of Venomous Animals and Toxins including Tropical Diseases*, **2018**, 24, 27-33.



Mitochondrial Imbalance Caused by Semi-Synthetic Derivatives of Licarin A in *Leishmania (L.) infantum*

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[2] Institute of Environmental, Chemical and Pharmaceutical Sciences, Universidade Federal de São Paulo, 09972-270, Brazil
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Visceral leishmaniasis affects more than 1.5 million people in 68 countries, with few and toxic therapeutic alternatives [1]. Considering the promising antitrypanosomal of activity of the neolignan licarin A, isolated from leaves of *Nectandra oppositifolia* (Lauraceae), this compound was used as scaffold for the semi-synthesis of O-methyl (**P1**), O-acetyl (**P2**), O-allyl (**P3**), and 5-allyl (**P4**) derivatives [2]. Compounds **P1 – P4** were evaluated against *Leishmania (L.) infantum* and for mammalian cytotoxicity. No haemolytic activity was detected in BALB/c mice erythrocytes in the range of 1.6 to 200 µM. Three compounds exhibited activity against the intracellular amastigotes, with IC_{50} values of 1.5 µM (**P2**), 12.7 µM (**P3**) and 6.9 µM (**P4**) and selectivity indexes (SI) of 134.2, 3.9 and 28.9, respectively. *In silico* studies for drug-likeness and ADME, suggested a good oral bioavailability, with no alerts for *pan-assay interference compounds* (PAINS). Using spectrofluorimetric techniques and flow cytometry, the lethal action of the compounds was investigated in promastigotes. In the presence of the fluorescent probe Sytox Green, the compounds showed no alteration of the plasma membrane permeability of the parasite. The independent incubation with compounds **P2**, **P3** and **P4** with the promastigotes, resulted in a mitochondrial membrane depolarization without alterations of the reactive oxygen species (ROS) levels. A time-dependent increase of Ca^{2+} levels was observed after the

treatment with compounds **P2**, **P3** and **P4**. We hypothesize that the mitochondrial imbalance observed in *Leishmania* after treatment with these semi-synthetic derivatives may be ascribed to the Ca^{2+} release from the intracellular pools, leading to a toxic effect in the parasite. Further studies are still needed to explore the mechanism of action of these licarin A derivatives. Supported by FAPESP (2020/03637-3, 2017/50333-7)

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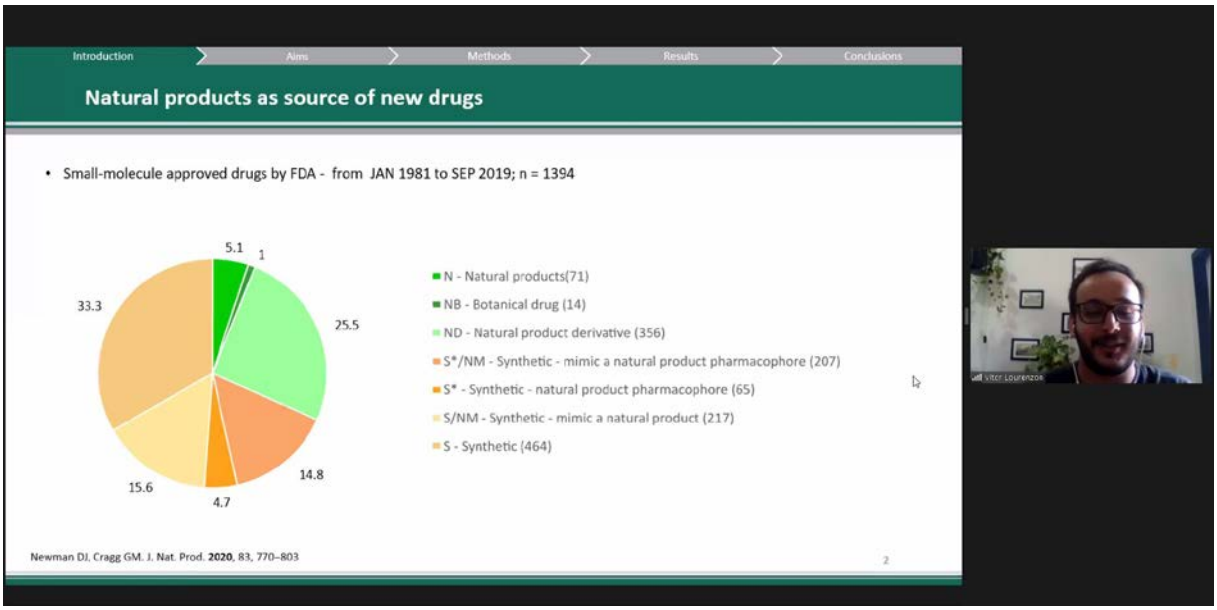

Biosynthetic pathway of cyphomycin, an antileishmanial polyketide produced by ant-associated *Streptomyces*

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Natural products play an important role in the discovery of new bioactive compounds, inspiring the development of new drugs. In this context, microorganisms have a remarkable contribution since they are capable of producing several secondary metabolites active against microbial pathogens.[1] The association with microorganisms proved to be an important evolutionary advantage for the most diverse living beings such as plants, insects, and animals, which can use these compounds as chemical defenses against different pathogens.[2] Our research group at FCFRP-USP has focused on bacterial symbionts of fungus-growing ants (Attine tribe) as sources of antimicrobial agents. Recently, three structurally related polyketides were isolated and identified from the *Streptomyces* sp. ISID311, an actinobacterium isolated from winged males of *Cyphomyrmex* fungus-growing ants.[3,4] One new compound, named cyphomycin, was identified together with previously reported caniferolide C and GT-35. The compounds showed high antifungal activity against different strains of the specialized entomopathogenic fungus *Escovopsis,* and also, *in vitro* and *in vivo* activity against human fungal pathogens. The compounds also showed high antiparasitic activity against both the intracellular amastigote and promastigote forms of *Leishmania donovani,* a human parasite that causes Leishmaniasis, a neglected tropical disease. Thus, this project aims to elucidate the biosynthetic

pathway of cyphomycin and analogs, by *in silico* analysis of the complete genome for biosynthetic gene cluster (BGC) identification. Cyphomycin type 1 polyketide synthase BGC is 220 kb in length and contains 83 genes, 25 of them were directly related to the structure biosynthesis. The polyketide chain is biosynthesized by seven polyketide synthases (PKS), and modified by CYP450 that promote specific oxidations. The sugars L-axenose and D-aminocetose are biosynthesized from glucose-1-phosphate by the action of nine enzymes. Finally, alkylnaphtoquinone biosynthesis starts with an intermediate from the menaquinone pathway and is completed by the product of three other genes.[5]

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In vitro antileishmanial properties of essential oil extracted from *Citrus limon* from Tunisia

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Essential oil of *Citrus limon* is used for its many biological properties such as antioxidant, antibacterial, and anti-inflammatory. These properties are interesting for the treatment of skin diseases [1]. Lemon pulp was used to treat pimples and wrinkles or to soften facial skin [2,3]. On the other hand, limonene, one of the major bioactive molecules in *Citrus limon* plant, exhibited an interesting *in vitro* antileishmanial activity against *L. major* and *L. amazonensis* parasites [4]. For the best of our knowledge, the antileishmanial potentialities of whole essential oil extracted from *Citrus l.* is investigated for the first time. This study is part of the current trend of research for new antileishmanial therapeutic molecules, less or nontoxic and more efficient compared to existing commercial drugs that have multiple adverse side effects, such as nausea, anaemia, fever, and possibly drug resistance [5]. Moreover, the molecule screening of medicinal plants could be an interesting way to identify alternative natural, cheaper, and safe sources of antileishmanial molecules.

The antileishmanial activity of essential oil extracted from leaves of Tunisian *Citrus limon* was assessed *in vitro* against two forms of *Leishmania major* parasite, i.e., the axenic amastigote and the intramacrophage forms [6]. The *Citrus l.* essential oil displayed an interesting activity against both forms of *L. major* with IC_{50} value at 4.2 ± 1.3 µg/mL for intramacrophage amastigotes form. Interestingly, this activity was close to that of miltefosine. Besides, low cytotoxicity with high selectivity

index was proved for this essential oil, revealing its safety for macrophages. This interesting result could be linked, either to the activity of major compounds of the essential oil, such as citral compounds, or to synergetic effects between different compounds. The lipophilic properties of terpenes were shown in literature to contribute to the disruption of parasite intracellular metabolic pathways.

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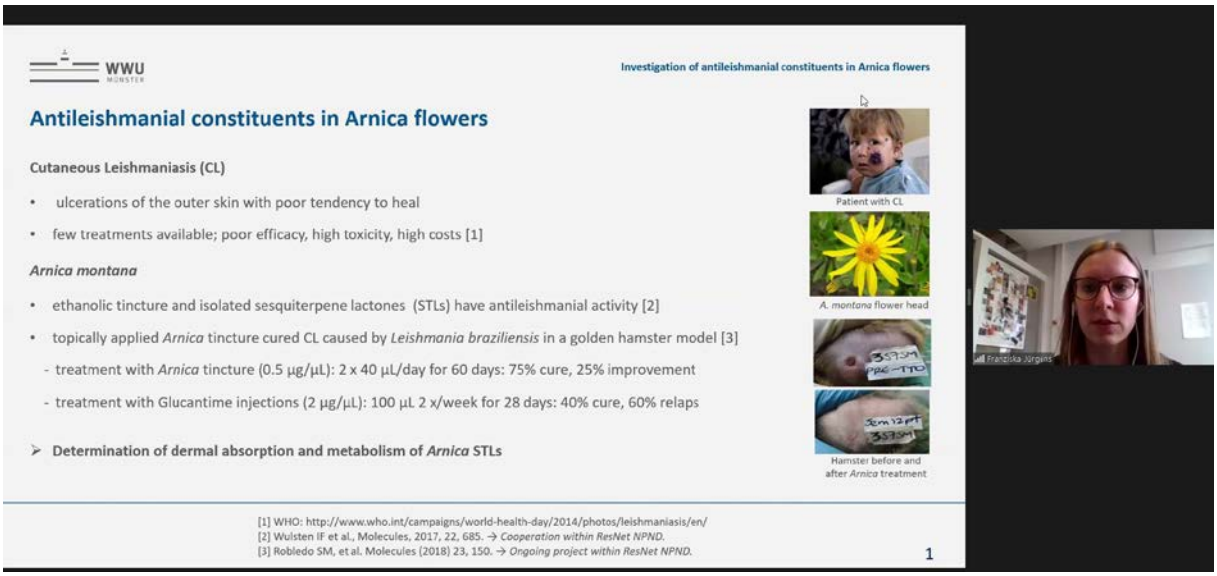

Investigation of antileishmanial constituents in *Arnica* flowers

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Arnica flowers contain sesquiterpene lactones of the helenalin type which are known for their anti-inflammatory activity [1]. These secondary metabolites are also active against various *Leishmania ssp.* including pathogens of cutaneous Leishmaniasis (CL) like *L. braziliensis* [2]. Skin ulcers and lesions bearing the risk of serious disabilities characterize this neglected tropical disease [3]. Since the standard drugs for CL treatment have toxic side effects and poor patient compliance, new efficacious, safe and practical therapies are urgently needed [3].

In a previous study [2], an ethanolic tincture of *Arnica montana* flowers showed curative activity in experimental CL in a golden hamster model. In comparison with Glucantime®, a standard antileishmanial drug, even more hamsters were cured with the Arnica tincture treatment.

To enable the use of Arnica constituents for the treatment of CL, biopharmaceutical studies to determine the ADME profile are required. Among them, *in vitro* metabolism and absorption studies are essential.

In this work, selected Arnica sesquiterpene lactones were incubated with pig liver microsomes. As a result, phase I and phase II metabolites were identified using UHPLC-QTOF MS/MS experiments.

In addition to that, an *in vitro* skin absorption study with skin samples of Göttingen minipigs was performed. In this experiment, Arnica sesquiterpene lactones permeated into the skin as well as into the acceptor fluid. Furthermore, main metabolites as known from the *in vitro* metabolism studies were found in the acceptor fluids and skin extracts of the *in vitro* skin absorption study.

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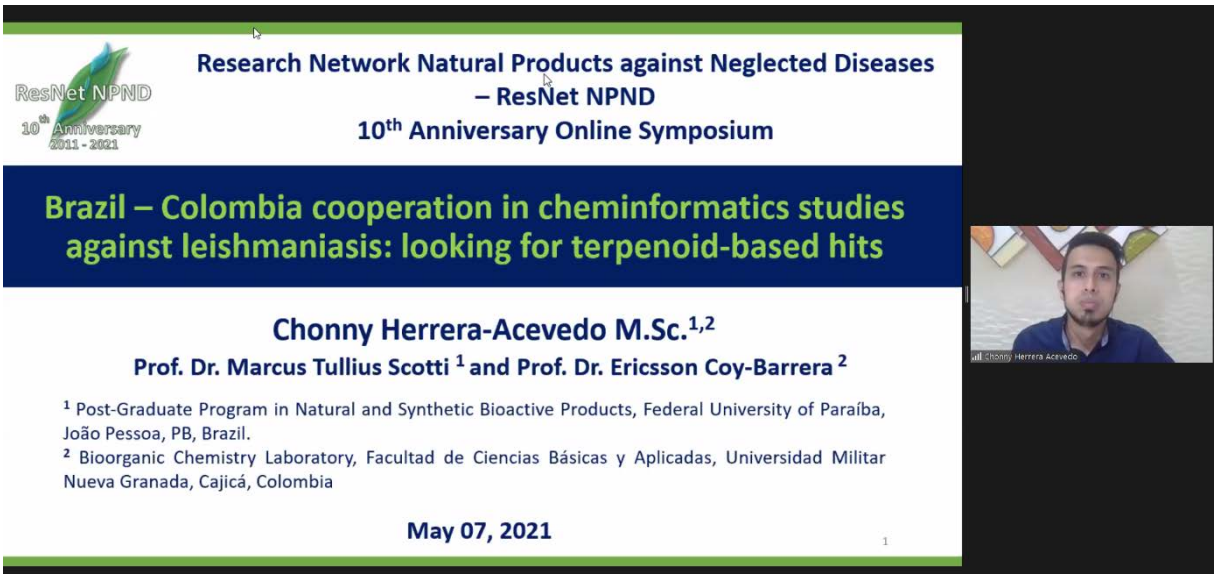

Brazil – Colombia cooperation in cheminformatics studies against leishmaniasis: looking for terpenoid-based hits.

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Within the framework of a regional cooperation (Brazil-Colombia) between ResNet NPND members, a series of studies in cheminformatics have been performed, looking for terpenoid-based hits against leishmaniasis, a group of neglected tropical diseases that affect more than one million people worldwide [1]. Initially, an *in-house* database of 360 kauranes (tetracyclic diterpenes) was generated, and a combined ligand- and structure-based virtual screening (VS) approach was performed to select potential inhibitors of *Leishmania major* (*Lm*) pteridine reductase I (*Lm*PTR1). For the ligand-based virtual screening, a machine learning classification model was built from the ChEMBL dataset (657 structures) which was classified as either active or inactive (binary classification). Sensitivity values of 78.1% and 82.6 % and specificity values of 72.7% and 73.7%, were obtained for the cross-validation and test sets, respectively. Only 7 from 360 structures were classified as active (ligand-based probability value [*LB*] ≥ 0.5), with structures **135** (2β-hydroxy-menth-6-en-5β-yl *ent*-kaurenoate) and **302 (**3α-cinnamoyloxy-*ent*-kaur-16-en-19-oic acid) representing two of the best-ranked kauranes, with *LB* values of 0.57 and 0.54, respectively. These two kauranes were employed to verify the validity of the VS approach through *Lm*PTR1 enzyme inhibition assay. The half-maximal inhibitory concentration (IC_{50}) values of selected bioactive compounds were below 10 μM, as predicted in the classification model. A compound structurally related to **302**, 3α-*p*-coumaroyloxy-*ent*-kaur-16-en-19-oic acid (**302a**),

was also synthesized and showed the highest activity against *Lm*PTR1. Finally, molecular docking calculations and molecular dynamics simulations were performed for the VS-selected, most-active kauranes within the active sites of PTR1 hybrid models, generated from three *Leishmania* species that are known to cause cutaneous leishmaniasis in the new world (i.e., *L. braziliensis*, *L. panamensis,* and *L. amazonensis*) [2,3] to explore the targeting potential of these kauranes to other species-dependent variants of this enzyme.

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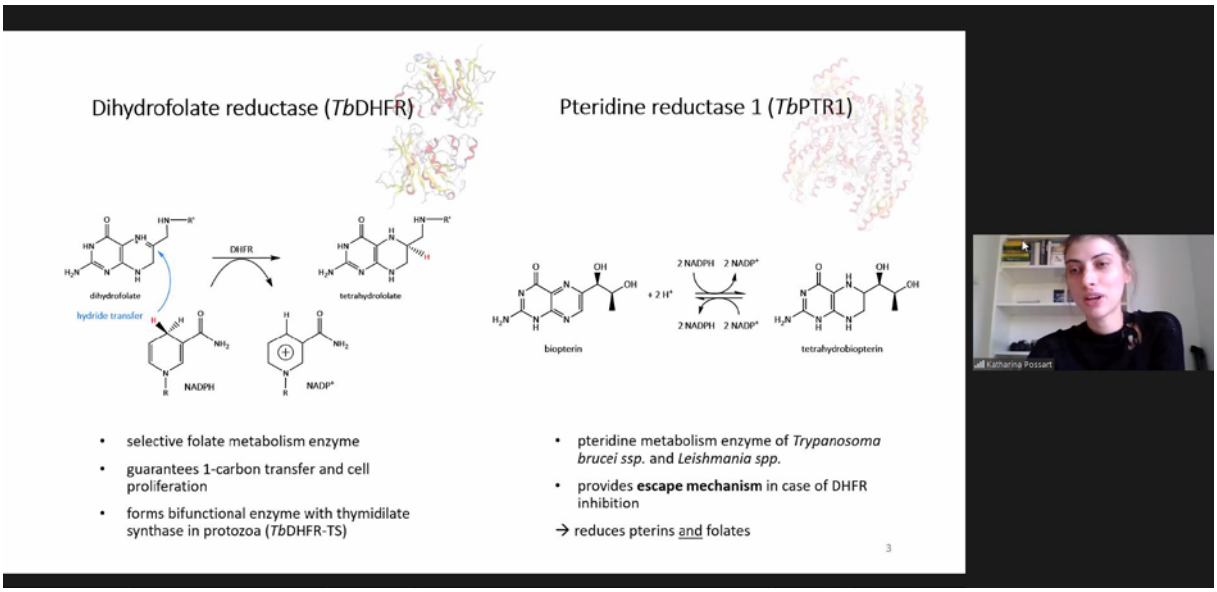

Rational search for natural inhibitors of the *Trypanosoma brucei* pteridine metabolism

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The blood parasite *Trypanosoma brucei* is the cause of human African trypanosomiasis (HAT) as well as the cattle disease “Nagana” which still represent a massive health burden for many countries in Africa [1]. So far, the majority of the available treatment options is accompanied by harmful side effects and constantly threatened by newly emerging drug resistances due to the highly adaptive nature of the parasites [2].

Since trypanosomes are auxotrophic for folate, their pteridine metabolism provides a promising target for an innovative chemotherapeutic treatment. They are equipped with a unique corresponding enzyme system consisting of the bifunctional dihydrofolate reductase-thymidylate synthase (*Tb*DHFR-TS) and the pteridine reductase 1 (*Tb*PTR1). Previously, gene knockout experiments with PTR1 null mutants have underlined the importance of these enzymes for parasite survival [3].

The aim of this project is the identification of natural products with a dual inhibitory activity against the *Tb*DHFR and *Tb*PTR1. A multi-step *in silico* procedure was employed to pre-select promising candidates with an inhibitory potential against the targeted enzymes. Ligand- and target-based pharmacophore models were created based on existing 3D protein structures of both enzymes. They were applied to perform a virtual screening of various natural product databases (about 15000 compounds in total) followed by a multi-step docking process. So far, almost 50 selected *in silico* hits that were available from various sources were tested in a spectrophotometric inhibition assay against

*Tb*PTR1 followed by the determination of their half maximum inhibitory concentration (IC_{50}) as well as their kinetic mode of inhibition. As a result, 21 natural products with different scaffolds were identified as new hits against *Tb*PTR1 (IC_{50} < 50 µM), of which the strongest inhibitor displayed an IC_{50} below 10µM. Further work aimed at recombinant expression of *Tb*DHFR and establishment of an assay for inhibition of this enzyme is in progress.

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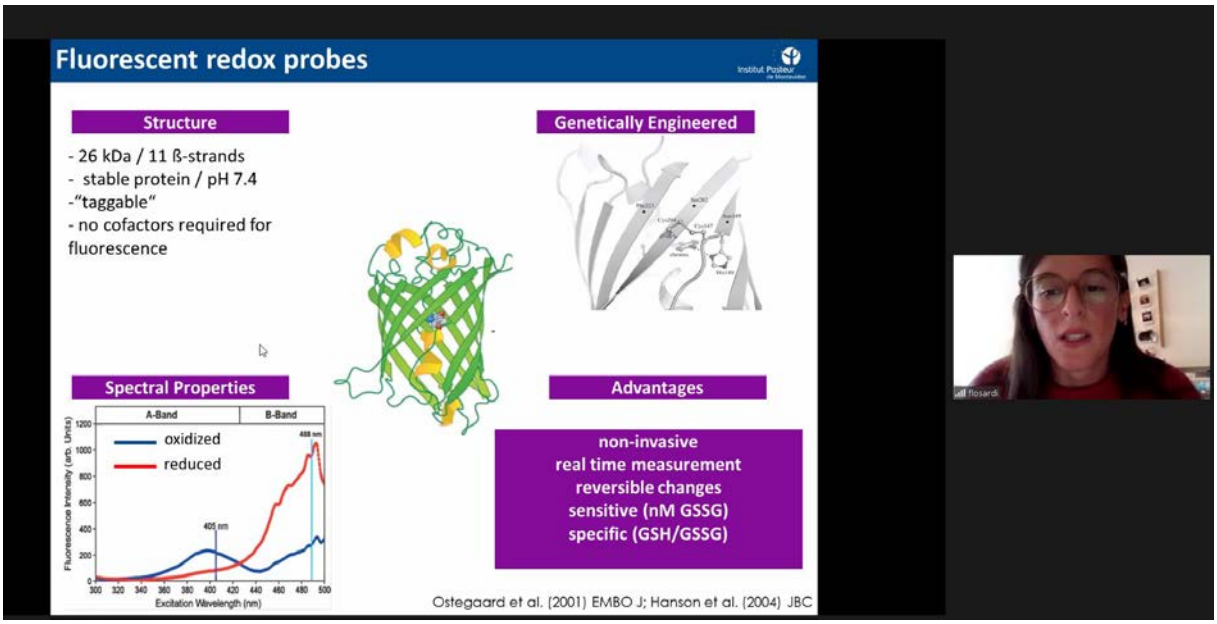

Development of redox reporter cell lines of pathogenic trypanosomatids and insights into the mechanism of action of natural sesquiterpene lactones

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The thiol-dependent redox metabolism of trypanosomatids has been shown to be critical for parasite survival and persistence in the mammalian host, and for contributing to (pro)drug activation and resistance mechanisms. The quantitative monitoring of intracellular redox changes in real-time demands the development of reliable reporter cell lines with redox-sensitive and -specific fluorescent biosensors.

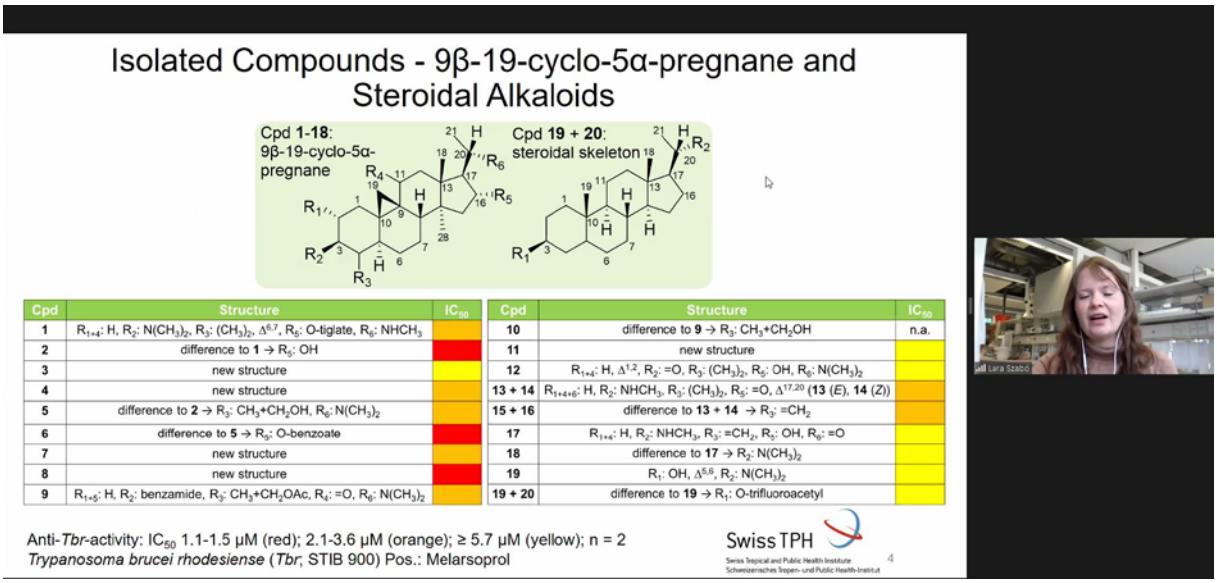
Here we report the generation of stable transgenic cell lines of pathogenic trypanosomatids expressing a novel redox biosensor consisting of a glutaredoxin (Grx) that catalyzes the oxidation-reduction of a C-terminally linked redox-sensitive GFP (roGFP2). Furthermore, these cell lines were employed to investigate the mode of action of a small subset of sesquiterpene lactones (SL). This type of compounds is present in several plants and corals, and is a rich source of drugs.

Recombinant Grx-roGFP2 proved to be sensitive and specific to detect changes in the amount of reduced and oxidized low molecular weight thiols. Transgenic cell lines of infective *Trypanosoma brucei brucei* and *Leishmania infantum* expressing Grx-roGFP2 were characterized by flow cytometry. The

reporter cell lines showed a concentration-dependent response to different oxidants, which was reversible by incubation with DTT, a permeant reducing agent.

Seven SL were tested against *T. brucei* and *L. infantum*, with the most potent displaying EC_{50} values of 0.3 and 6 µM, respectively. SL harbour a α,β-unsaturated carbonyl group that is highly electrophilic and prone to react with the thiol group present in cysteine residues. The modification of redox active cysteines may have important consequences in cell redox homeostasis. Interestingly, the redox reporter parasites revealed that not all SL with antiproliferative activity against trypanosomatids induced oxidative stress as part of their killing mechanism. Although of different magnitude, three SL generated intracellular oxidation in both parasite species, suggesting common molecular target(s).

We demonstrated the potential of redox reporter trypanosomatids for targeted-drug discovery studies.



Antitrypanosomal Alkaloids of *Buxus sempervirens* L.

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Human African Trypanosomiasis (HAT) is considered as one of the most challenging neglected tropical diseases (NTD) due to its limited therapeutic options and high fatality rate. Hence, the development of innovative and effective antitrypanosomal drugs is indispensable. Natural products provide a vast field of new lead structures that play a vital role in the drug discovery and development process.

In a previous study [1], a bioactivity-guided isolation has led to the isolation of Cyclovirobuxeine-B from a *Buxus sempervirens* L. (common box; Buxaceae) leaf extract. This *nor*-cycloartane alkaloid showed promising and selective in vitro activity against *Trypanosoma brucei rhodesiense* (*Tbr*), causative agent of HAT.

Our purpose is the isolation of additional alkaloids from the leaves of *B. sempervirens* L. to search for further related compounds with strong antitrypanosomal activity and to establish structure-activity relationships.

As a result, 23 alkaloids were isolated from *B. sempervirens* L., including eight new natural products and one compound first described for this plant. During the isolation procedure, an alkaloid fraction was obtained from the crude dichloromethane extract by acid-base extraction, which was subsequently separated into 20 fractions by centrifugal partition chromatography (CPC). The CPC fractions exhibited a wide range of antitrypanosomal activity in the in vitro assay. To obtain an

indication of compounds responsible for the differences in bioactivity between the fractions a comprehensive UHPLC/+ESI-QqTOF-MS/MS analysis of the fractions coupled with partial least squares (PLS) regression modelling was performed.

In conclusion, all isolated alkaloids were tested for activity against *Tbr*. Several of them displayed promising in vitro activity against *Tbr* in a sub-micromolar range. The PLS model highlighted likewise these constituents as responsible for the antitrypanosomal activity along with further alkaloids, which were identified by dereplication.

To summarize, the alkaloids from *B*. *sempervirens* L. have the potential to serve as an antitrypanosomal lead structure.

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Discovering the potential of the secondary metabolites from *Malouetia tamaquarina* (Apocynaceae) and its endophytes against neglected tropical diseases

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More than 1 billion people that live in tropical and subtropical areas, under poverty conditions are affected by Neglected Tropical Diseases (NTDs), [1]. Some NTDs caused by protozoa, such as Chagas disease and Human African Trypanosomiasis (HAT), have a huge impact in Latin American and African countries [2]. In addition, the low efficacy and high toxicity of the current drugs and the lack of vaccines against trypanosomiasis, reinforce the urgency for new drugs [3, 4].

Natural products isolated from plants and their endophytic microorganisms can be an excellent alternative for new drug candidates [5]. *Malouetia tamaquarina* is a plant that belongs to the same tribe of *Holarrhena africana* within the family Apocynaceae, whose compounds have shown strong activity against *Trypanosoma brucei rhodesiense*, one of the pathogens of HAT [6]. Since *M. tamaquarina* is known to conaing aminosteroid-type alkaloids like *H. africana* [7], this plant is likely to present a strong antitrypanosomal activity.

Endophytic microorganisms are bacteria or fungi that live inside healthy plant cells without causing any symptom of disease [5]. Many secondary metabolites isolated from endophytes have presented good antiprotozoal activity [8]. However, there are no reports described about endophytes of *M. tamaquarina.* The aim of this work is

therefore, to investigate the potential of *M. tamaquarina* and its endophytes against NTDs. Leaves and bark of *M. tamaquarina* were collected in the Amazon forest, the endophytic microorganisms were isolated and their biological activities, such as antimicrobial activities and antiprotozoal activities were assessed. The crude extracts of the endophytes showed antimicrobial activity against the pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* and also antiprotozoal activity against *Trypanosoma cruzi* Tulahuen *lac*Z and *Leishmania donovani*, demonstrating the potential of the endophytes of *M. tamaquarina* against NTDs. The isolation and identification of biologically active secondary metabolites are currently underway.

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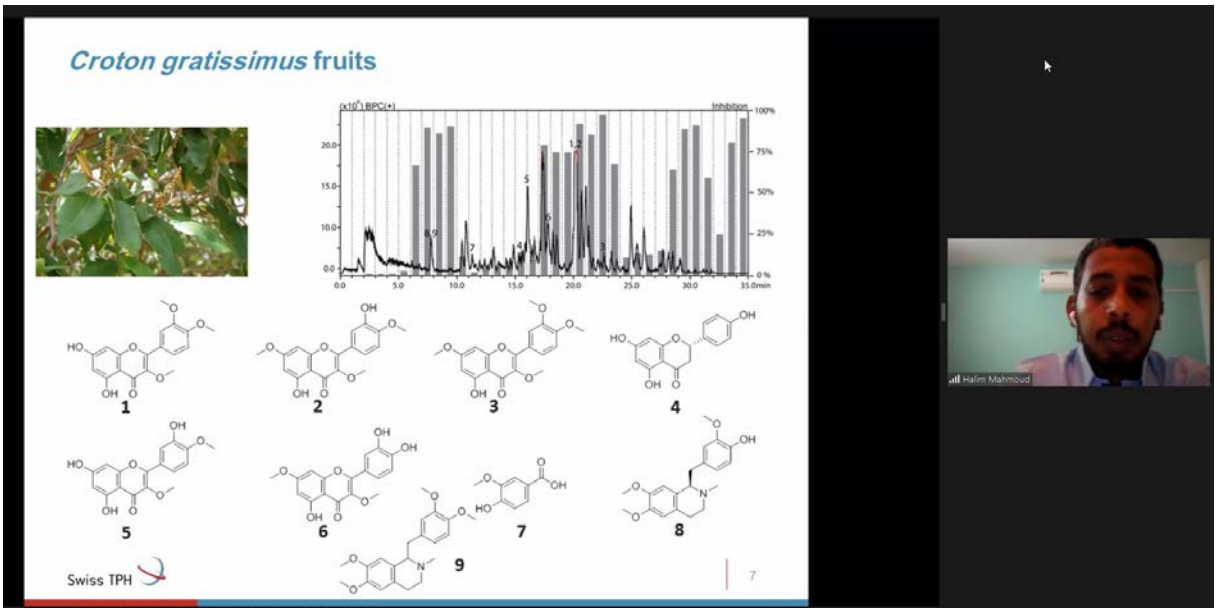

HPLC-Based Activity profiling of Sudanese Medicinal Plants for Natural Compounds against Tropical Parasitic Diseases

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Tropical parasitic diseases such as malaria, human African trypanosomiasis, Chagas disease, mycetoma, and leishmaniasis affect more than a billion people worldwide and have devastating consequences. There is no vaccine for any of these diseases, and the current drugs are problematic given their serious adverse effects and the emergence of drug-resistant parasites [1]. Natural products have in many instances provided new leads to combat neglected tropical diseases [2]. This work aims to compile Sudanese medicinal plants, validate their antiprotozoal activities, and identify active molecules.

A library of 235 plant extracts was prepared from over 60 plants used in Sudanese traditional medicine, and it was assessed for *in vitro* antiprotozoal activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi, Leishmania donovani,* and *Plasmodium falciparum* [3]. Plants that displayed interesting activities, namely *Croton gratissimus* (Euphorbiaceae), *Cuscuta hyalina* (Convolvulaceae), and *Haplophyllum tuberculatum* (Rutaceae) , were further pursued using HPLC-based activity profiling approach.

HPLC-based activity profiling of *Croton gratissimus* allowed the identification of flavonoids, mainly quercetin derivatives, as responsible for the antileishmanial activity of the chloroform fraction of the crude ethanolic extract [4]. Phytochemical characterization of *Cuscuta hyalina* led to the isolation of a

unique flavonoid, pseudosemiglabrin, for the first time from *Cuscuta* species [4]. From *Haplophyllum tuberculatum,* lignans, amides, and steroidal saponins were isolated for the first time from *Haplophyllum* species and the family Rutaceae [5]. The lignan, Nectandrin B, exhibited the highest activity against *L. donovani* (IC_{50} 4.5 µM) and the highest selectivity index (25.5).

Several compounds were selected for screening against mycetoma. Among them, magnolol possessed the highest activity (MIC of 15 µM) and selectivity (SI of 4.9) against *Madurella mycetomatis*.

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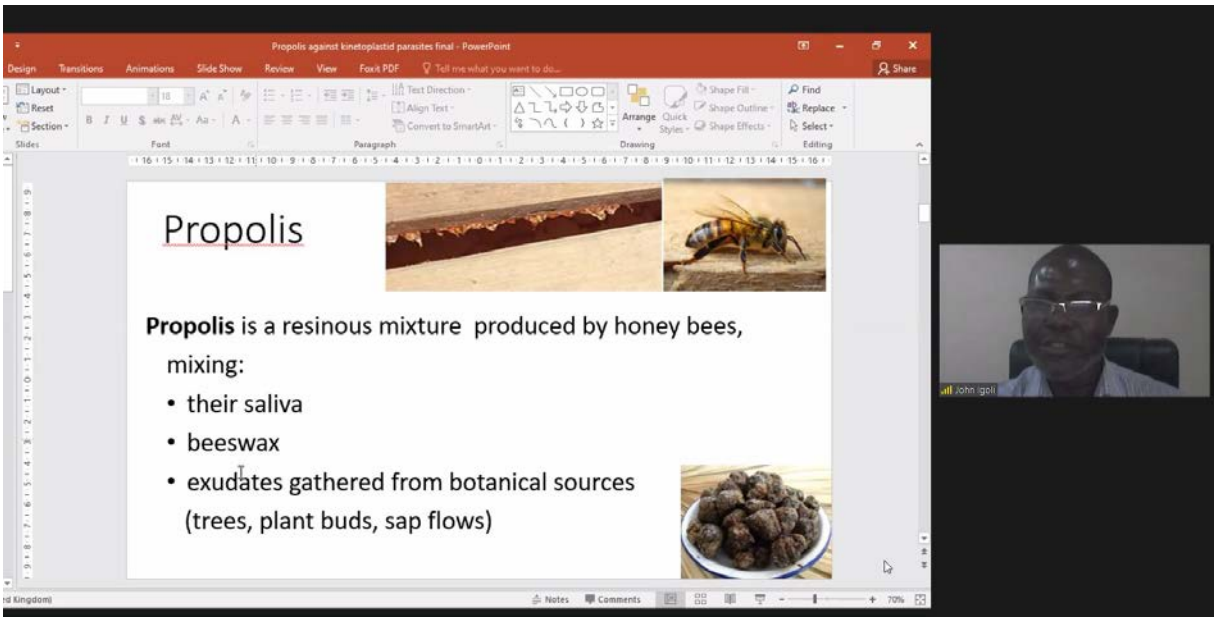

Propolis against kinetoplastid parasites

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Propolis, its extracts and products have been involved in human health for ages. Bees produce propolis possibly for their own health benefits, but also as a means of securing their hives, protecting as well as sanitizing their hive environment. Exploitation of propolis extracts and compounds have revealed bioactivities beneficial to man and bees. Propolis contains many natural compounds collected by foraging bees from tree buds and exudates in the vicinity of their hives. These compounds have been shown to be carefully selected to address or control microorganisms and parasites that invade the hive or otherwise infect the bee colony. Among these bee pathogens are protozoan parasites *Crithidia bombi, C. mellificae and Lotmaria passim.* They are all kinetoplastid parasites and related to important disease agents of humans and livestock, such as *Trypanosoma brucei* spp, *Trypanosoma congolense*, *Trypanosoma cruzi* and *Leishmania* spp. As such it is expected that propolis would be active against human and animal parasites. The first assay of propolis compounds against these parasites [1] showed that propolis compounds had anti-kinetoplastid activity. Subsequently studies [2-4] have confirmed this both for known and novel compounds isolated from propolis. It can thus be hypothesised that when bees suffer from or have threat of parasites in their hives, they produce propolis containing antiparasitic compounds. It is therefore pertinent to review and discuss the anti-kinetoplastid activity

of compounds isolated and characterized from bee propolis to guide further studies and contribute to drug discovery against these parasites are the agents of some of the most neglected tropical diseases.

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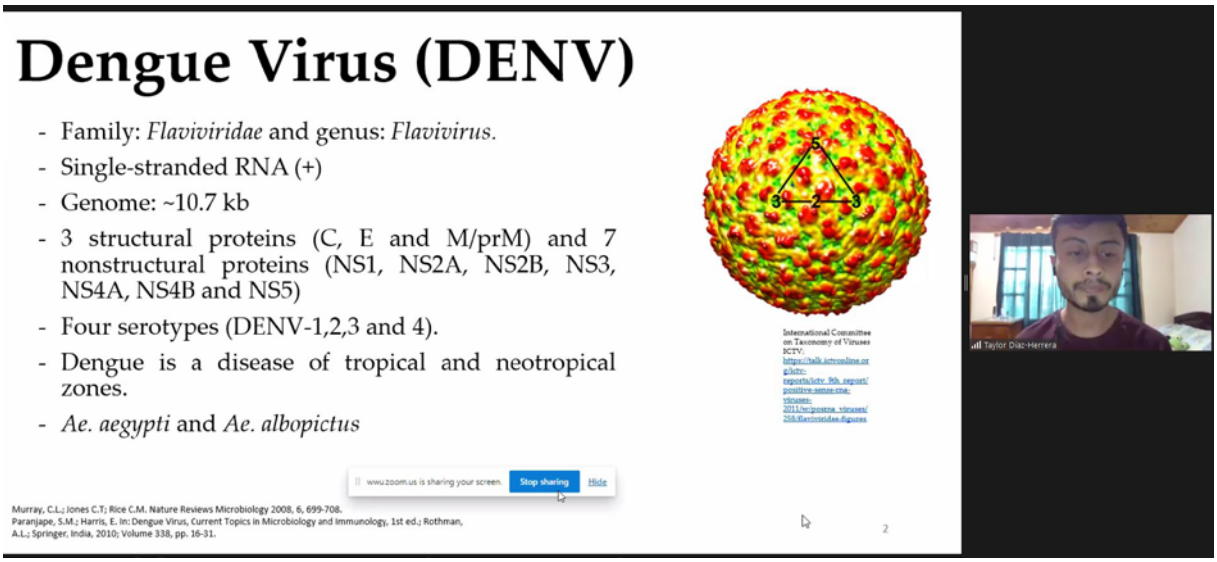

Anti-Dengue Activity of *Phyllanthus urinaria* (L.) against Four DENV Serotypes

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Dengue is the most important arthropod-borne viral disease in tropical countries, caused by the dengue virus (DENV) [1]. Currently, there is not an effective tetravalent vaccine or specific treatment to control or prevent DENV infections. Alternatives accepted for the control of this virus show disadvantages such as the absence of unified doses of the vaccine/treatments [2]. However, research on plant naturally-occurring anti-dengue phenolic-like compounds can be an alternative facing such a problem [3]. Thus, as part of our research on natural products against neglected diseases, the *in vitro* virucidal effect of an ethanolic extract of leafflower [*Phyllanthus urinaria* (L.)] was studied against four DENV serotypes as well as the electrostatic interactions between annotated phytoconstituents and the hydrophobic binding site of envelope protein of DENV by molecular docking. The chemical composition of the ethanolic extract was determined by UHPLC-QTOF MS/MS. Antioxidant capacity, total phenolic and total flavonoid contents were also established. The virucidal activity was evaluated against dengue (DENV-1, 2, 3 and 4) with different concentrations (0.19-25 µg/mL) of *P. urinaria* extract titled in LLC-MK2 cells. Extract exhibited a virucidal effect against DENV-1 and 2 at different levels following a dose-response behavior. Therefore, *P. urinaria* showed anti-DENV effect using the previously standardized cell culture. On the other hand, fifteen phenolics-related features were then pinpointed in the test *P. urinaria* extract. The molecular docking revealed important key interactions between *P. urinaria* compounds and the hydrophobic pocket of the DENV E protein, so the binding mode was

computationally-predicted to potentially inhibiting the virus entry. Therefore, *P. urinaria* would represent a promising source of anti-viral phenolics against DENV to be employed in further R&D.

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In vitro and *in vivo* anthelmintic activity of *Stevia multiaristata* extract on *Echinococcus granulosus*

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Cystic echinococcosis (CE) is a worldwide zoonotic disease caused by the larval stage of the parasite *Echinococcus granulosus,* which causes long-term infections in humans and animals, being a serious public health problem [1]. Albendazole (ABZ), the main drug used against CE, has undesirable side effects and its efficacy is about 50% [2]. Thus, n*ew* treatment alternatives are urgently needed. Plants from *Stevia* genus (Asteraceae) are a potential source of antiprotozoal and antimicrobial compounds. The antiparasitic activity of extracts from different *Stevia* species has recently been demonstrated on *Trypanosoma cruzi* and *Leishmania braziliensis* [3]. In the current study, we demonstrated the *in vitro* efficacy of the *Stevia multiaristata* extract against protoscoleces and murine cyst of *E. granulosus.* Moreover, we investigated the clinical efficacy of the *S. multiaristata* extract in a murine model of CE. *S. multiaristata* extract caused a rapid decrease on protoscoleces viability, reaching 0% at day 6 with the concentration of 100 µg/ml. At the ultrastructural level, protoscoleces treated with 100 µg/ml experimented a total loss of morphology at day 3 post-incubation. Loss of turgidity was detected in 95% of cysts incubated during 2 days with 10 µg/ml and the collapse of the germinal layer was observed in 60% of cysts treated with 5 µg/ml during 4 days. Although the median weight of cysts recovered

from ABZ treated mice was lower than the observed in the control group, no significant differences were found ($P > 0.05$). In contrast, *S. multiaristata* treatment caused a significant decrease in the weight of the cysts compared with control group ($P < 0.05$). In conclusion, *S. multiaristata* extract demonstrated a marked *in vitro* and *in vivo* effect against *E. granulosus* larval stage.

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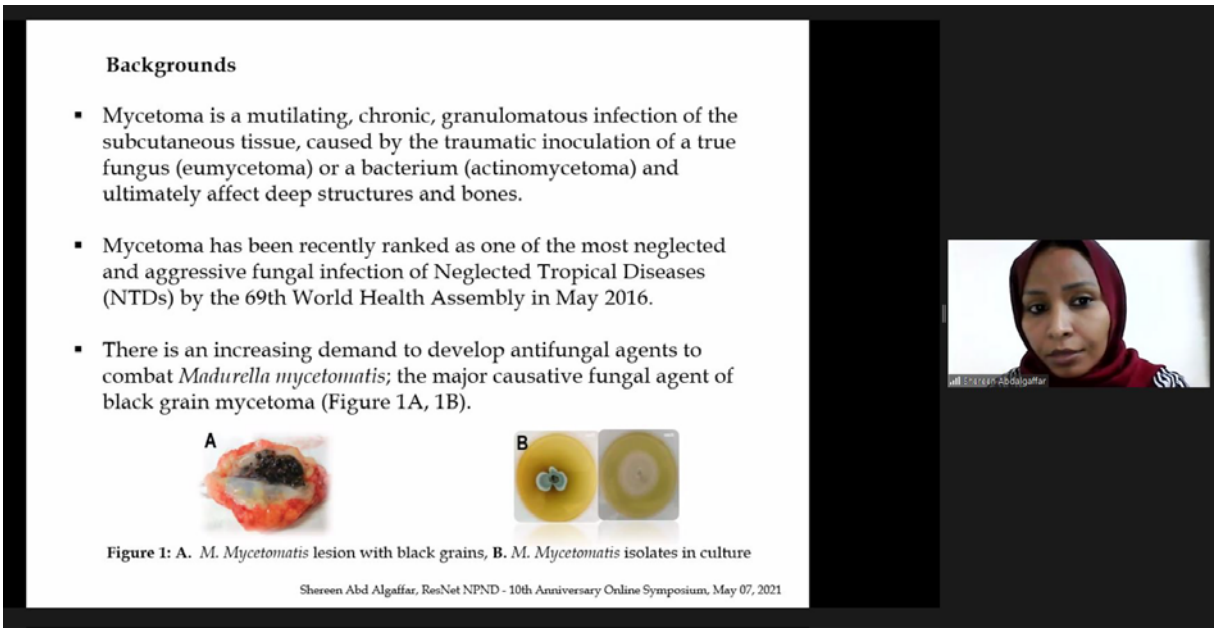

Drug Discovery of Natural Bioactive Molecules against *Madurella mycetomatis*- One of the Most Neglected Diseases

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Mycetoma is a mutilating, chronic, granulomatous infection of the subcutaneous tissue, caused by the traumatic inoculation of a fungus (eumycetoma) or a bacterium (actinomycetoma) and ultimately affect deep structures and bones [1]. Currently, the drug discovery pipeline for mycetoma is basically blank. Fosravuconazole; which was originally discovered for the treatment of Chagas disease, is currently going through a proof of concept superiority in a clinical trial in Sudan.

Our recently published *in vitro* susceptibility assay for *M. mycetomatis* is reproducible, less costly compared with *in vitro* XTT assay and can be used for testing the antifungal activity of any extract, fraction, or compound and thus is ideal for drug discovery; it offers an extra advantage of visual endpoint reading without resorting to spectrophotometric measurements, as well as the flexibility with homemade plate layouts, especially in endemic settings [2].

Employing resazurin assay, screening of more than 70 essential oils (EOs) of taxonomically diverse medicinal plants, 100 plant extracts/ fractions, isolated pure compounds revealed promising hits, for instance *Matricaria chamomile, Geigeria alata,* and *Croton zambesicus* against both eumycetoma and actinomycetoma. Nevertheless, there is a pressing demand for simple, inexpensive *in vivo* model to study disease burden, toxicity, and efficacy of promising bioactive compounds to work alongside the

in vitro and *in silico* models. This prompt us to develop in-house an *in vivo Galleria mellonella* larvae model [3] to speed up the drug discovery process against NTDs. None of the EOs which exhibited MIC (0.125- 0.0078%v/v) had shown toxicity to *Galleria* larvae at 1 and 0.5%v/v concentrations with ≥80% survival except for 1% v/v *Croton zambesicus* (66.6%). Efficacy studies on *Galleria* model are well undergoing for those EOs showing no *in vivo* toxicity.

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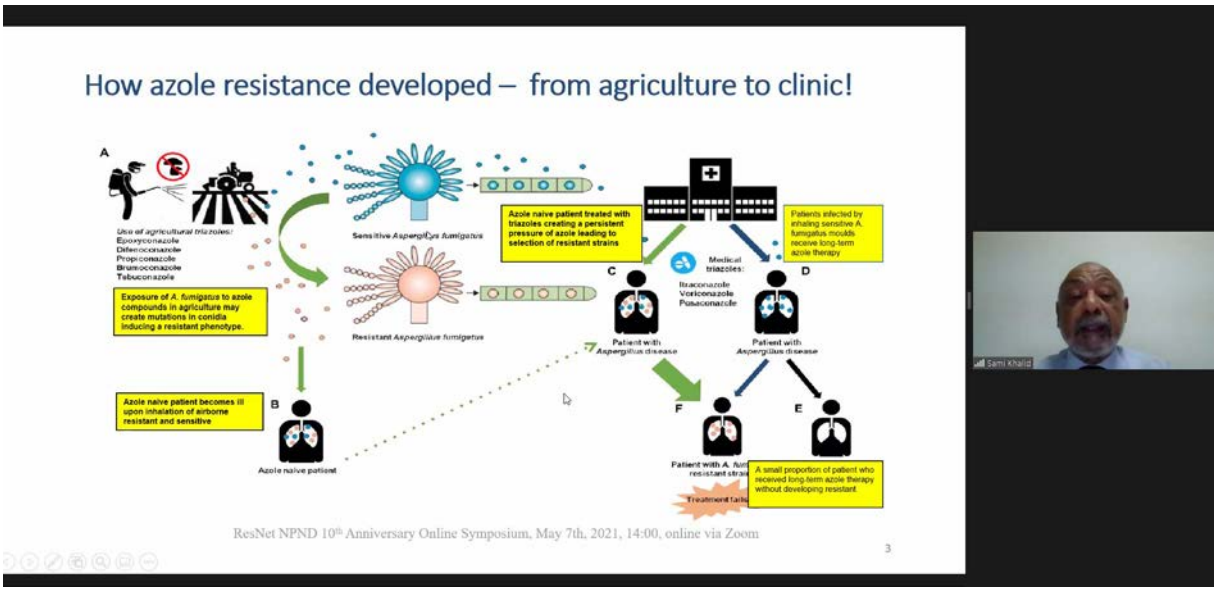

Network highlight lecture

Mycetoma Drug Discovery Research Platform Conslidates the Concept of Networking

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Mycetoma is a chronic, progressively destructive inflammatory disease acquired by traumatic inoculation of certain fungi (Eumycetoma) or bacteria (Actinomycetoma) into the subcutaneous tissue. It has been finally considered as one of the most neglected disease in 2016 by the WHO and added to the list of the neglected tropical diseases (NTDs) well after its adoption by the 2nd ResNet NPND Workshop in Rio de Janeiro as an integral component of the NTDs in 2014 [1].

The presentation intends to showcase the interest and activity of the members of the network on mycetoma during the last years. Hence, several members of the network expressed their interest to provide their samples to be screened by our *in vitro* resazurin-based susceptibility assay which was developed in 2014 against *Madurella mycetomatis* and recently been published after its full validation [2]. An exponential interest in mycetoma has been later demonstrated during the last years by members of the network and 552 samples, including extracts, fractions, and pure compounds were received from Brazil, Germany, Argentina, and United States. Among them 474 (85.9%) were screened against the two mycetomal types with 202 (42.6%) revealed appreciable activity. The exhibited antimycetomal activity of processed samples against eumycetoma was much less 41 (8.6%) compared with their actinomycetoma 65 (13.7%) counterparts, whiles 96 (20.3%) samples exhibited activities against both types. Several of the secondary metabolites received as pure compounds (139) including terpenoids, polyphenols, and alkaloids revealed appreciable 28 (20.3%) activity. Essential oils screened

(111) showed remarkable activity 71 (63.9%) against mycetoma types. Total synthetic compounds screened 45 with 12 (26.7%) compounds exhibited activity solely against actinomycetoma.

Compounds revealed exceptionally good *in vitro* activity are usually considered to be subjected to *Galleria mellonella* larvae *in vitro* toxicity and efficacy assays.

Undoubtedly, that ResNet NPND provided a forum for members to foster professional relationships and the Mycetoma Drug Discovery Research Platform intends to consolidate one of the cardinal mission of our network.

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