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HELENA MANNOCHIO RUSSO

Metabolomic tools for chemosystematic studies and identification of bioactive natural products in Malpighiaceae species

Thesis submitted to the Institute of Chemistry, São Paulo University (UNESP), as part of the requirements for obtaining the title of Doctor in Chemistry.

Supervisor: Profª. Drª. Vanderlan da Silva Bolzani

Araraquara
2022

Russo, Helena Mannochio
R969m Metabolomic tools for chemosystematic studies and identification of bioactive natural products in Malpighiaceae species / Helena Mannochio Russo – Araraquara: [s.n.], 2022
285 p.: il.

Thesis (doctor) – Universidade Estadual Paulista, Instituto de Química
Advisor: Vanderlan da Silva Bolzani

1. Metabolites. 2. Mass spectrometry. 3. Chemotaxonomy. 4. Evolution. 5. Nitro compounds. I. Title.

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HELENA MANNOCHIO RUSSO

Ferramentas metabolômicas para estudos quimiossistemáticos e identificação de produtos naturais bioativos em espécies de Malpighiaceae

Tese apresentada ao Instituto de Química, Universidade Estadual Paulista (UNESP), como parte dos requisitos para obtenção do título de Doutora em Química

Orientadora: Profª. Drª. Vanderlan da Silva Bolzani

Araraquara
2022

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Araraquara

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: "Ferramentas metabolômicas para estudos quimiossistemáticos e identificação de produtos naturais bioativos em espécies de Malpighiaceae"

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Curriculum Vitae

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2) Education

2018 – 2022 Ph.D. in Chemistry

Institute of Chemistry, São Paulo State University (UNESP), Araraquara, Brazil.

Title: Metabolomic tools for chemosystematic studies and identification of bioactive natural products in Malpighiaceae species.

Supervisor: Profª. Drª. Vanderlan da Silva Bolzani.

Scholarship: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Process number: 142014/2018-4.

2019 – 2020 Research Internship (8 months).

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego (UCSD), California, United States.

Title: Molecular networking: a rational and innovative strategy for chemosystematic studies of Malpighiacae and identification of biologically active natural products.

Supervisor: Prof. Dr. Pieter Dorrestein.

Scholarship: Fulbright Program.

2015 – 2018 Master's in Chemistry

Institute of Chemistry, São Paulo State University (UNESP), Araraquara, Brazil.

Title: Evaluation of the chemical profile of the methanolic extract of *Niedenzuella multiglandulosa* leaves: isolation, characterization and identification of the bioactive compounds.

Supervisor: Profª. Drª. Vanderlan da Silva Bolzani (Co-supervisor: Prof. Dr. Emerson Ferreira Queiroz).

Scholarship: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Process number: 152431/2015-2.

Research Internship: School of Pharmacy Genève-Lausanne. University of Geneva (UNIGE), Geneva, Switzerland (5 months).

2010 – 2015 Bachelor in Chemistry

Institute of Chemistry, University of Campinas (UNICAMP), Campinas, Brazil.

Undergraduate research project: Application of continuous flow chemistry in the synthesis of natural products and derivatives with potential antitumor activity.

Supervisor: Prof. Dr. Julio Cezar Pastre.

Scholarship: Fundo de Apoio ao Ensino, à Pesquisa e à Extensão (FAEPEX).

2013 Undergraduate Research Internship

School of Pharmacy Genève-Lausanne. University of Geneva (UNIGE), Geneva, Switzerland (6 months).

Supervisor: Prof. Dr. Emerson Ferreira Queiroz.

Title: Phytochemical investigation of cashew (*Anacardium occidentale*).

3) Scientific awards

- **2022:** Selected to participate in the 71st Lindau Nobel Laureate Meeting (dedicated to Chemistry), in Lindau (Germany) – June 26th to July 1st, 2022.

- **2022:** Selected to attend the Baden-Würtemberg International Post Conference Programme of the 71st Lindau Nobel Laureate Meeting – July 1st to July 8th, 2022.
- **2022:** CAS Future Leaders in Chemistry, American Chemical Society.
- **2022:** Women in Science – UNESP. Category: Graduate student – Exact and Earth Sciences. Dean of Graduate Studies (PROPG/UNESP).
- **2019:** Fulbright Doctoral Dissertation Research Award (DDRA): 2019-2020. Fulbright Commission.
- **2019:** Panel awarded by the Natural Products Chemistry Division – 42nd Annual Meeting of the Brazilian Chemical Society.
- **2017:** Best presentation award in the VIII Symposium of Thermal Analysis (Brazil).

4) Publications in scientific journals

- BAUERMEISTER, ANELIZE; **MANNOCHIO-RUSSO, HELENA**; COSTA-LOTUFO, LETÍCIA V.; JARMUSCH, ALAN K.; DORRESTEIN, PIETER C. Mass spectrometry-based metabolomics in microbiome investigations. *Nature Reviews Microbiology*, v. 20, p. 143-160, **2022**.
- **MANNOCHIO-RUSSO, H.**; DE ALMEIDA, R.; NUNES, W. D.; BUENO, P. C. P.; CARABALLO-RODRÍGUEZ, A. M.; BAUERMEISTER, A.; DORRESTEIN, P.; BOLZANI, V. S. Untargeted metabolomics sheds light on the diversity of major classes of secondary metabolites in the Malpighiaceae botanical family. *Frontiers in Plant Science*, v. 13, p. 854842, **2022**.
- FERRARI, A. B. S.; OLIVEIRA, G. A.; **RUSSO, H. M.;** BERTOZO, L. C.; BOLZANI, V. S.; ZIED, D. C.; XIMENES, V. F.; ZERAIK, M. L. *Pleurotus ostreatus* and *Agaricus subrufescens*: investigation of chemical composition and antioxidant properties of these mushrooms cultivated with different handmade and commercial supplements. *International Journal Of Food Science And Technology*, v. 56, p. 452-460, **2021**.
- RIBEIRO, D. C.*; **RUSSO, H. M.*;** Fraige, K.; ZERAIK, M. L.; NOGUEIRA, C. R.; SILVA, P. B.; CODO, A. C.; CALIXTO, G. M. F.; MEDEIROS, A. I.; CHORILLI, M.; BOLZANI, V. S. Bioactive Bioflavonoids from *Platonia insignis* (Bacuri) Residues as Added Value Compounds. *Journal Of The Brazilian Chemical Society*, p. 786-799, **2021**.

- FERRARI, A. B. S.; MARCHEAFAVE, G. G.; **MANNOCHIO-RUSSO, H.;** BOLZANI, VANDERLAN S.; ZIED, D. C.; SCARMINIO, I. S.; ZERAIK, M. L. Chemical composition and chromatographic fingerprint of three strains of *Agaricus subrufescens* cultivated with handmade and commercial supplements. *Food Chemistry*, v. 363, p. 130227, **2021**.
- SCHMID, R. PETRAS, D. NOTHIAS, L-F. WANG, M. ARON, A. JAGELS, A. TSUGAWA, H. RAINER, J. GARCIA-ALOY, M. DUHRKOP, K. KORF, A. PLUSKAL, T. KAMENIK, Z. JARMUSCH, A. CARABALLO-RODRIGUEZ, A. WELDON, K. NOTHIAS-ESPOSITO, M. AKSENOV, A. BAUERMEISTER, A. ORIO, A. A. GRUNDMANN, C. VARGAS, F. KOESTER, I. GAUGLITZ, J. GENTRY, E. , HOVELMANN, Y. KALININA, S. PENDERGRAFT, M. PANITCHPAKDI, M. TEHAN, R. GOUELLEC, A. L. **MANNOCHIO RUSSO, HELENA** ARNDT, B. HUBNER, F. HAYEN, H. ZHI, H. RAFFATELLU, M. PRATHER, K. ALUWIHARE, L. BOCKER, S. MCPHAIL, K. HUMPF, H. KARST, U. DORRESTEIN, P. C. Ion identity molecular networking for mass spectrometry-based metabolomics in the GNPS environment. *Nature Communications*, v. 12, p. 3832, **2021**.
- GONÇALVES NUNES, WILHAN DONIZETE*; **MANNOCHIO RUSSO, HELENA***; DA SILVA BOLZANI, VANDERLAN; CAIRES, FLÁVIO JUNIOR. Thermal characterization and compounds identification of commercial *Stevia rebaudiana* Bertoni sweeteners and thermal degradation products at high temperatures by TG-DSC, IR and LC-MS/MS. *Journal of Thermal Analysis And Calorimetry*, v. 146, p. 1149-1155, **2021**.
- **RUSSO, H. M.**; QUEIROZ, E. F.; MARCOURT, L.; RUTZ, A.; ALLARD, P-M.; ALMEIDA, R. F.; CARVALHO, N. M.; WOLFENDER, J. L.; BOLZANI, V. S. Phytochemical analysis of the methanolic leaves extract of *Niedenzuella multiglandulosa* (Malpighiaceae), a plant species toxic to cattle in Brazil. *Phytochemistry Letters*, v. 37, p. 10-16, **2020**.
- SCUPINARI, T.*; **RUSSO, H. M.*;** FERRARI, A. B. S.; BOLZANI, V. S.; DIAS, W. P.; NUNES, E. O.; HOFFMANN-CAMPO, C. B.; ZERAIK, M. L. *Crotalaria spectabilis* as a source of pyrrolizidine alkaloids and phenolic compounds: HPLC-MS/MS dereplication and monocrotaline quantification of seed and leaf extracts. *Phytochemical Analysis*, v. 31, p. 747-755, **2020**.

- **MANNOCHIO-RUSSO, H.**; BUENO, P. C. P.; BAUERMEISTER, A.; ALMEIDA, R. F.; DORRESTEIN, P. C.; CAVALHEIRO, A. J.; BOLZANI, V. S. Can Statistical Evaluation Tools for Chromatographic Method Development Assist in the Natural Products Workflow? A Case Study on Selected Species of the Plant Family Malpighiaceae. *Journal Of Natural Products*, p. 3239-3249, **2020**.
- QUEIROZ, SUZANA APARECIDA S.; PINTO, MERI EMILI F.; BOBEY, ANTONIO F.; **RUSSO, HELENA M.;** BATISTA, ANDREA N.L.; BATISTA, JOAO M.; CODO, ANA C.; MEDEIROS, ALEXANDRA I.; BOLZANI, VANDERLAN S. Diterpenoids with inhibitory activity of nitrite production from *Croton floribundus*. *Journal Of Ethnopharmacology*, v. 249, p. 112320, **2020**.
- MATUTINO BASTOS, TANIRA; **MANNOCHIO RUSSO, HELENA**; SILVIO MORETTI, NILMAR; SCHENKMAN, SERGIO; MARCOURT, LAURENCE; GUPTA, MAHABIR; WOLFENDER, JEAN-LUC; FERREIRA QUEIROZ, EMERSON; BOTELHO PEREIRA SOARES, MILENA. Chemical Constituents of *Anacardium occidentale* as Inhibitors of *Trypanosoma cruzi* Sirtuins. *Molecules*, v. 24, p. 1299, **2019**.
- VALLI, MARILIA; **RUSSO, HELENA MANNOCHIO**; PILON, ALAN CESAR; PINTO, MERI EMILI FERREIRA; DIAS, NATHALIA B.; FREIRE, RAFAEL TEIXEIRA; CASTRO-GAMBOA, IAN; BOLZANI, VANDERLAN da SILVA. Computational methods for NMR and MS for structure elucidation II: database resources and advanced methods. *Physical Sciences Reviews*, v. 4, p. 20180167, **2019**.
- VALLI, MARILIA; **RUSSO, HELENA MANNOCHIO**; PILON, ALAN CESAR; PINTO, MERI EMILI FERREIRA; DIAS, NATHALIA B.; FREIRE, RAFAEL TEIXEIRA; CASTRO-GAMBOA, IAN; BOLZANI, VANDERLAN da SILVA. Computational methods for NMR and MS for structure elucidation I: software for basic NMR. *Physical Sciences Reviews*, v. 4, p. 20180108, **2019**.
- **RUSSO, HELENA MANNOCHIO***; NUNES, WILHAN DONIZETE GONÇALVES*; DA SILVA BOLZANI, VANDERLAN; IONASHIRO, MASSAO; CAIRES, FLÁVIO JUNIOR. Thermoanalytical and spectroscopic characteristics of young and old leaves powder and methanolic extracts of *Niedenzuella multiglandulosa*. *Journal of Thermal Analysis and Calorimetry*, v. 132, p. 771-776, **2018**.

- MEIRA, CÁSSIO S.; FILHO, JOSÉ MAURÍCIO DOS SANTOS; SOUSA, CAROLINE C.; ANJOS, PÂMELA S.; CERQUEIRA, JÉSSICA V.; NETO, HUMBERTO A. DIAS; DA SILVEIRA, RAFAEL G.; **RUSSO, HELENA M.;** WOLFENDER, JEAN-LUC; QUEIROZ, EMERSON F.; MOREIRA, DIOGO R.M.; SOARES, MILENA B.P. Structural design, synthesis and substituent effect of hydrazone-*N*-acylhydrazones reveal potent immunomodulatory agents. *Bioorganic & Medicinal Chemistry*, v. 26, p. 1971-1985, **2018**.
- MARILIA VALLI; **RUSSO, HELENA M.;** BOLZANI, V. S. The potential contribution of the natural products from Brazilian biodiversity to bioeconomy. *Anais Da Academia Brasileira De Ciências*, v. 90, p. 763-778, **2018**.

*Authors contributed equally.

5) Articles accepted for publication in scientific journals

- SILVA, G. L.; CAMPIDELLI, M.; FERRARI, A. B. S.; **MANNOCHIO-RUSSO, H.**; Fraige, K.; DAMETTO, A. C.; BOLZANI, V.; ZERAIK, M. L. In vitro antiglycation and antioxidant properties of *Eugenia pyriformis* leaves and fruits. *Natural Product Research*, **2022**. http://dx.doi.org/10.1080/14786419.2021.2005049.
- TAKAYAMA, K. S.; MONTEIRO, M. C.; SAITO, P.; PINTO, I. C.; NAKANO, C. T.; MARTINEZ, R. M.; THOMAZ, D. V.; VERRI JR, W. A.; BARACAT, M. M.; ARAKAWA, N. S.; **RUSSO, H. M.**; ZERAIK, M. L.; CASAGRANDE, R.; COUTO, R. O.; GEORGETTI, S. R. *Rosmarinus officinalis* extract-loaded emulgel prevents UVB irradiation damage to the skin. *Anais Da Academia Brasileira De Ciências*, **2022**.

6) Articles submitted in scientific journals

- SILVA, DULCE HELENA SIQUEIRA; **MANNOCHIO-RUSSO, HELENA**; LAGO, JOÃO HENRIQUE GHILARDI; BUENO, PAULA CAROLINA PIRES; MEDINA, REBECA PREVIATE; BOLZANI, VANDERLAN DA SILVA; VILEGAS, WAGNER, NUNES, WILHAN DONIZETE GONÇALVES. Bioprospecting as a strategy for conservation and sustainable use of the Brazilian Flora. *Biota Neotropica*, **2022**.
- ZANATTA, ANA C.; BORGES, MAIARA S.; **MANNOCHIO-RUSSO, HELENA**; HEREDIA-VIEIRA, SILVIA CRISTINA; SANTOS, LOURDES CAMPANER; RINALDO, DANIEL; VILEGAS, WAGNER. Green chromatography as a novel

alternative for the quality control of *Serjania marginata* Casar. leaves. *Microchemical Journal*, **2022**.

7) Book chapters

- MARILIA VALLI; **MANNOCHIO RUSSO, HELENA**; PILON, ALAN CESAR; PINTO, M. E. F.; DIAS, NATHALIA B.; FREIRE, RAFAEL TEIXEIRA; CASTRO-GAMBOA, IAN; BOLZANI, V. S. Computational methods for NMR and MS for structure elucidation II: database resources and advanced methods. In: Fidele Ntie-Kang. (Org.). Chemoinformatics of Natural Products - Volume 1 Fundamental Concepts. 1ed. Berlim: De Gruyter, **2020**, p. 205-228.
- MARILIA VALLI; **MANNOCHIO RUSSO, HELENA**; PILON, ALAN CESAR; PINTO, M. E. F.; DIAS, NATHALIA B.; FREIRE, RAFAEL TEIXEIRA; CASTRO-GAMBOA, IAN; BOLZANI, V. S. Computational methods for NMR and MS for structure elucidation I: software for basic NMR. In: Fidele Ntie-Kang. (Org.). Chemoinformatics of Natural Products - Volume 1 Fundamental Concepts. 1ed. Berlim: De Gruyter, **2020**, p. 177-204.

8) Participations in scientific events during the doctorate period

- **MANNOCHIO-RUSSO, H.**; BUENO, P. C. P.; BAUERMEISTER, A.; ALMEIDA, R. F.; DORRESTEIN, P. C.; CAVALHEIRO, A. J.; BOLZANI, V. S. Use of Statistical Tools and Automation to Accelerate the Natural Products Workflow – A Malpighiaceae Case Study. In: II Bio.Natural-Bioactive Natural Products Research Meeting, Lisbon, Portugal, **2021**. Invited talk (online).
- **MANNOCHIO-RUSSO, H.**; ALMEIDA, R. F.; NUNES, W. D. G.; ALBERNAZ, L.; ESPINDOLA, L. S.; BOLZANI, V. S. Screening of Malpighiaceae Plant Extracts Against *Aedes aegypti* Larvae and Metabolomic Investigation of the Most Active Species. In: 8th Brazilian Conference on Natural Products, Goiânia/GO, Brazil, **2021**. Poster presentation (online).
- RAMOS, A. V. G.; **MANNOCHIO-RUSSO, H.**; CABRAL, M. R. P.; SAHM, B.; LOTUFO, L.; CARMO, M.; SARRAGIOTTO, M. H.; BALDOQUI, D. C. Antitumoral activity of *Vernonanthura cuneifolia* and targeted isolation of sesquiterpene lactones based on Molecular Networking. In: 8th Brazilian Conference on Natural Products, Goiânia/GO, Brazil, **2021**. Poster presentation (online).

- **MANNOCHIO-RUSSO, H.**; ALMEIDA, R. F.; BUENO, P. C. P.; BAUERMEISTER, A.; CARABALLO-RODRIGUEZ, A. M.; DORRESTEIN, P. C.; BOLZANI, V. Untargeted metabolomics sheds light on the secondary metabolism of Malpighiaceae family. In: GA – 69th Annual Meeting, Bonn, Germany, **2021**. Poster presentation (online).
- **RUSSO, H. M.**; BUENO, P.; BAUERMEISTER, A.; ALMEIDA, R. F.; DORRESTEIN, P. C.; CAVALHEIRO, A. J.; BOLZANI, V. S. Experimental design applied in UPLC-DAD method development of Malpighiaceae species extracts and metabolite identification. In: American Chemical Society Spring 2020 National Meeting, Philadelphia, United States **2020**. Poster presentation (online).
- ZANATTA, A. C.; **RUSSO, H. M.**; MEDEIROS, R. D.; RINALDO, D.; VILEGAS, W. Perfil químico e screening de atividades biológicas para os extratos de *Byrsonima intermedia*, *Serjania marginata* e *Terminalia catappa*. In: XII Simpósio Brasileiro de Farmacognosia, Petrópolis/RJ, Brazil, **2019**. Poster presentation.
- **RUSSO, H. M.**; ALVES, N. F.; RIBEIRO, D. C.; ZOCOLO, G. J.; VALLI, M.; BOLZANI, V. S. Identificação de metabólitos secundários com atividade anti-glicante em extratos da espécie *Talisia esculenta*. In: XII Simpósio Brasileiro de Farmacognosia, Petrópolis/RJ, Brazil, **2019**. Poster presentation.
- **RUSSO, H. M.**; SCUPINARI, T.; FERRARI, A. B. S.; NUNES, E. O.; BOLZANI, V. S.; ZERAIK, M. L. Chemical profile of *Crotalaria spectabilis* extracts and evaluation of nematode control in *Heterodera glycines*. In: 42a Reunião Anual da Sociedade Brasileira de Química, Joinville/SC, Brazil, **2019**. Poster presentation.
- **RUSSO, H. M.**; ALMEIDA, R. F.; CAVALHEIRO, A. J.; BOLZANI, V. S. Experimental planning for UPLC method development of hydroalcoholic extracts from Malpighiaceae species. In: XVII Latin American Symposium on Chromatography and Related Techniques, Aracaju/SE, Brazil, **2019**. Poster presentation.
- FERRARI, A. B. S.; **RUSSO, H. M.**; ZIED, D. C.; BOLZANI, V. S.; ZERAIK, M. L. Dereplication by LC-MS/MS of three strains of *Agaricus subrufescens* grown with different supplements. In: XVII Latin American Symposium on Chromatography and Related Techniques, Aracaju/SE, Brazil, **2019**. Poster presentation.
- FERRARI, A. B. S.; SCUPINARI, T.; RUSSO, H. M.; DIAS, W. P.; NUNES, E. O.; ZERAIK, M. L. LC-MS/MS analysis of *Crotalaria spectabilis* leaves and seeds

extracts and its nematicidal and nematostatic effects. In: XVII Latin American Symposium on Chromatography and Related Techniques, Aracaju/SE, Brazil, **2019**. Poster and oral presentation.

- RUSSO, HELENA MANNOCHIO; RIBEIRO, D. C.; Fraige, K.; ZERAIK, M. L.; BOLZANI, V. S. Search for natural products of economic interest in fruit residues of *Platonia insignis*: bioactivity evaluation and structural characterization. In: XXV Encontro de Química da Região Sul (SBQSul), Londrina/PR, Brazil, **2018**. Oral presentation.

9) Short-term courses

- **2021:** Machine Learning in Chemometrics, 6 hours. Lecturer: Prof. Dr. Peter Harrington. V Escola de Inverno de Quimiometria. Online.
- **2021:** Multivariate regression: basic concepts and application examples, 8 hours. Lecturer: Prof. Dr. Dmitry Kirsanov. Escola de Inverno de Quimiometria. Online.
- **2018:** Morfologia e sistemática de Malpighiaceae Neotropical, 30 hours. Lecturer: Dr. Rafael Felipe de Almeida. Universidade Federal de Minas Gerais (UFMG).

10) Organization and/or presentations of workshops and courses

- **2021:** Lecturer of the short-term course “Basic concepts of mass spectrometry and use of the Global Natural Products Social Molecular Networking (GNPS) platform”. 10 hours. Online. Faculty of Pharmaceutical Sciences, Federal University of Alfenas (UNIFAL). 35 participants.
- **2021:** Workshop – “Molecular Networking with GC-MS data (in Portuguese)”. 4 hours. Online. Estimated audience: 80 people.
- **2020:** Workshop – “Feature-Based Molecular Networking (in Portuguese)”. 4 hours. Online. Estimated audience: 200 people
- **2020:** Workshop – “Molecular Networking (in Portuguese)”. 2 hours. Online. Estimated audience: 450 people.
- **2020:** Workshop – “Automated tools for developing chromatographic methods: Fusion QbD”. 2 hours. Online. Estimated audience: 90 people.
- **2019:** Workshop – “Young Researchers: future leaders in the academic and industrial sectors”. 4 hours. Workshop held in the 42a Reunião Anual da Sociedade Brasileira de Química, Joinville/SC, Brazil. Estimated audience: 80 people.

11) Scientific supervisions

- **2018-2019:** Anna Beatriz Sabino Ferrari (Department of Chemistry, State University of Londrina, UEL). Master research project, “Chemical analysis of *Agaricus subrufescens* mushroom grown with different handmade and commercial supplements”. Supervisor: Profª. Drª. Maria Luiza Zeraik.
- **2018-2019:** Giselle Lopes da Silva (Department of Chemistry, State University of Londrina, UEL). Master research project, “Phytochemical study and antiglycant and antioxidant evaluation of fruits *Psidium cattleianum* var. lucidum”. Supervisor: Profª. Drª. Maria Luiza Zeraik.
- **2019:** Natalia Felix Alves (Chemical Engineering, São Paulo State University, UNESP). Undergraduate research project, “Bioguided study of *Talisia esculenta* for the identification of secondary metabolites inhibitors of advanced glycation”. Supervisor: Profª. Drª. Vanderlan da Silva Bolzani.
- **2018:** Dayane Castro Ribeiro (Faculty of Pharmaceutical Sciences, São Paulo State University, UNESP). Undergraduate research project, “Evaluation of the potential of Brazilian fruits for the development of safe functional food of economic interest”. Supervisor: Profª. Drª. Vanderlan da Silva Bolzani.

12) Scientific affiliations

- Member of the Brazilian Chemical Society (SBQ, 2019 – Present) – Member of the SBQ Young Researchers Committee (JP-SBQ) since its creation.
- Member of the Metabolomics Society (2021 – Present).
- Member of the International Young Chemists Network (IYCN/IUPAC) – Brazilian delegate (2021 – Present).

13) Other relevant information

- Member of the editorial board of Revista Virtual de Química (RVSBQ) – Special Edition coordinated by JP-SBQ: “Science Developed by Young Brazilian Researchers / A Ciência Desenvolvida por Jovens Pesquisadores Brasileiros”. **2021-2022**.
- Seven interviews granted to news outlets about the doctoral work focused on the search for natural substances to combat *Aedes aegypti*, **2021**.

I dedicate this thesis to my dear parents, Elisa and Valter, for their unconditional support in my personal and professional life. My journey to this point is also a victory for them!

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and thanks to (in Portuguese):

Inicialmente, gostaria de agradecer aos meus amados pais, Elisa e Valter, por sempre terem me apoiado e servido de exemplo não só de carreira, mas para a vida. Sem vocês eu não teria conseguido chegar tão longe.

Ao meu marido e companheiro, Wilhan D. G. Nunes, que acompanha todos os meus passos e conquistas desde o mestrado. Ter você ao meu lado fez com que essa trajetória tenha sido mais leve. Obrigada pela ajuda e apoio, tanto no âmbito pessoal quanto no profissional! Eu te amo!

À minha orientadora, professora Dra. Vanderlan, por ter me aceitado como aluna de doutorado, pela orientação, confiança, por todas as oportunidades que me concedeu, e por todos os ensinamentos transmitidos durante todos esses anos. Tê-la como orientadora é, sem sombra de dúvidas, uma grande inspiração!

Ao professor Dr. Pieter Dorrestein, por ter me recebido em seu laboratório durante meu estágio de doutorado-sanduíche, por todas as oportunidades que me concedeu e por todos os conhecimentos transmitidos. A minha estadia no Dorrestein Lab foi muito além do que eu poderia imaginar!

Um agradecimento especial ao Dr. Rafael Felipe de Almeida, pelos ensinamentos transmitidos, amizade e pela excelente colaboração. Grande parte desse trabalho só foi possível de ser realizada devido à sua grande ajuda!

Às professoras Dra. Lorena Albernaz e Dra. Laila Espíndola pelos ensaios realizados com o *Aedes aegypti*, pela excelente colaboração, atenção, e por toda a paciência.

Outro agradecimento especial à professora Dra. Maria Luiza Zeraik, pela grande amizade de longa data e por todas as oportunidades de colaboração que me concedeu durante o período de doutorado! Aprendi e aprendo muito com você, obrigada pela amizade e confiança.

Aos doutores Nivaldo Boralle, Juliana Rodrigues, João Bronzel pelo excelente apoio técnico no laboratório de RMN e nos laboratórios do NuBBE. Obrigada pelo tempo dedicado às análises e treinamentos, eu aprendi muito com vocês.

À professora Dra. Paula Bueno, pela amizade e ensinamentos transmitidos. Ter sua colaboração em grande parte desse trabalho foi essencial para produzirmos um trabalho de qualidade. É uma parceria que quero levar para a vida!

À Dra. Marilia Valli, pela amizade, por todas as oportunidades de colaboração, e por ter me chamado para participar da JP-SBQ!

Ao professor Dr. Alberto Cavalheiro pelos ensinamentos transmitidos, pela colaboração e amizade.

Também gostaria de agradecer ao professor Dr. Craig Nelson e ao M.Sc. Sean Swift, excelentes colaboradores da University of Hawai‘i, pelos ensinamentos transmitidos e pela oportunidade de colaborar ativamente nos projetos de Waimea Bay.

Aos meus colegas e amigos do Bolzani Team, NuBBE, e UNESP: Suzana Queiroz, Meri Emili Pinto, Antonio Bobey, Luiz Dutra, Ana Letícia Pires, Andrés Peña, Maria do Carmo, Rafael Vieira, Rafael Viana, Marcus Vinicius, Naira Anhesine, Karina Fontanin, Ana Zanatta e muitos outros. Também um agradecimento especial ao Marlon Oliveira, que mesmo já tendo defendido a alguns anos, mantemos uma amizade sincera.

Aos colegas e amigos do Dorrestein Lab: Alan Jarmusch, Alexander Aksenov, Allegra Aron, Amina Bouslimani, Anelize Bauermeister, Anupriya Tripathi, Carlismari Grundmann, Daniel Petras, Emily Gentry, Julia Gautlitz, Mabel González, Mauricio Caraballo, Mingxun Wang, Morgan Panitchpakdi, Tiago Leão. Thank you for the instructions, help, collaboration and friendship! My stay in San Diego would not be the same without you all.

Ao programa Fulbright pela bolsa concedida, tornando possível o sonho de realizar o doutorado-sanduíche em San Diego, Estados Unidos. Além da bolsa concedida, a

experiência Fulbright como um todo foi muito gratificante e me permitiu conhecer pessoas incríveis de diversos lugares do mundo. Sou muito feliz em fazer arte da família Fulbright!

Aos servidores do Instituto de Química, em especial à Seção Técnica de Pós-Graduação e à Biblioteca pelo apoio, excelente trabalho e competência.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa de doutorado concedida (#142014/2018-4).

Aos órgãos de fomento à pesquisa, FAPESP e CAPES que financiaram direta ou indiretamente este trabalho.

Finalmente, a todos que contribuíram direta ou indiretamente para este trabalho.

“If I have seen further, it is by standing on the shoulders of giants”

– Isaac Newton

RESUMO

Historicamente, as plantas estão entre as fontes de produtos naturais mais investigadas, e contribuíram significativamente para o desenvolvimento do campo da química de produtos naturais. Metabólitos de plantas representam uma diversidade estrutural rica, com inúmeras atividades biológicas que inspiraram o desenvolvimento de diversos fármacos. O Brasil é considerado um *hotspot* de biodiversidade contendo milhares de espécies sem nenhuma informação química relatada até o momento, representando um vasto potencial para estudos químicos. O desenvolvimento contínuo de ferramentas e bancos de dados de bioinformática de produtos naturais pode ser de grande valia para auxiliar e acelerar estudos na área de produtos naturais no Brasil. Nesse contexto, a família botânica Malpighiaceae se destaca por apresentar uma grande diversidade de produtos naturais reportados na literatura, e ainda contém centenas de espécies quimicamente inexploradas. O presente trabalho tem como objetivo estudar plantas da família Malpighiaceae, com três principais objetivos: (1) desenvolver um método cromatográfico otimizado (em UHPLC-PDA) para análise de uma mistura de extratos de espécies da família Malpighiaceae (MIX); (2) realizar um estudo quimiossistemático baseado em análises de UHPLC-MS/MS de 137 espécies de Malpighiaceae baseado em metabolômica não direcionada, buscas espectrais, e em ferramentas de classificação *in silico* recentemente descritas; (3) buscar por compostos bioativos com atividade larvicida contra o *Aedes aegypti* usando uma abordagem metabolômica. Os resultados obtidos destacam o poder do uso da abordagem *Quality by Design* para desenvolver um método cromatográfico robusto de amostras muito complexas, além do uso da ferramenta *Molecular Networking*, que levou à anotação bem-sucedida de 61 compostos nos nove extratos usados para preparar o MIX. Além disso, as ferramentas bioinformáticas utilizadas nas investigações quimiotaxonômicas revelaram diversas classes químicas específicas de determinados clados ou gêneros, e foram usados para reconstruir a filogenia de Malpighiaceae. Esta análise forneceu classes de metabólitos identificados como homoplásticos (ou seja, não exclusivos) ou sinapomórficos (ou seja, exclusivos) para clados e gêneros, e permitiu um estudo químico evolutivo abrangente em Malpighiaceae. Finalmente, para a busca de compostos bioativos, uma triagem inicial revelou a espécie *Heteropterys umbellata* como a mais promissora, que foi selecionada para uma investigação química mais aprofundada. Foi possível determinar as diferenças nos perfis metabólicos de caules e folhas e de coletas realizadas em diferentes estados do bioma Cerrado (Minas Gerais e São Paulo). Um fracionamento bioguiado levou ao isolamento de nitropropanoil glicosídeos, uma classe incomum de metabólitos que apresentaram atividade larvicida de moderada a potente. Em conclusão, este estudo contribuiu para investigar a química de dezenas de espécies de Malpighiaceae que ainda não haviam sido estudadas até então. O estudo permitiu a análise das classes químicas em Malpighiaceae em um contexto quimioevolutivo, além de levar ao isolamento de compostos naturais incomuns por uma abordagem de base metabolômica. Esses resultados contribuíram para ampliar o conhecimento químico sobre Malpighiaceae, e os fluxos de trabalho seguidos podem ser reproduzidos em outros táxons para futuras investigações químicas.

Palavras-chave: *Quality by Design*; quimiotaxonomia; metabolômica de plantas; desreplicação; espectrometria de massas; evolução; nitrocompostos.

ABSTRACT

Historically, plants are among the most investigated sources of natural products, which substantially contributed to the development of the natural products field. Plant metabolites represent a rich structural diversity, with countless biological activities that have inspired the development of several drugs. Brazil is considered a megadiverse hotspot, containing thousands of species without any chemical information reported to date, representing a vast potential for chemical studies. The continuous development of natural product bioinformatics tools and databases can be of great value to assist and accelerate studies in the natural products field in Brazil. In this context, the Malpighiaceae botanical family stands out for presenting a wide diversity of natural products reported to date, but still containing hundreds of chemically underexplored species. This research aims to study plant species from the Malpighiaceae family, with three main objectives: (1) develop an optimized chromatographic method (UHPLC-PDA) for the analysis of a mixture of extracts from Malpighiaceae species (MIX); (2) perform a chemosystematic investigation based on UHPLC-MS/MS analysis of 137 Malpighiaceae species based on MS/MS untargeted metabolomics, spectral searches, and recently described *in silico* classification tools; (3) search for bioactive compounds with larvicidal activity against *Aedes aegypti* using an untargeted metabolomics approach. The results obtained highlight the power of using the Quality by Design approach to develop a robust chromatographic method of very complex samples, as well as of the Molecular Networking tool, which led to the successful annotation of 61 compounds in the nine extracts used to prepare the MIX. In addition, the bioinformatics tools used in the chemotaxonomic investigations revealed several clade or genera-specific chemical classes, which were successfully used to reconstruct the Malpighiaceae phylogeny. This analysis provided classes of metabolites identified as homoplastic (*i.e.*, non-exclusive) or synapomorphic (*i.e.*, exclusive) for clades and genera, and allowed a comprehensive evolutionary chemical study in Malpighiaceae. Finally, for the search of bioactive compounds, an initial screening revealed *Heteropterys umbellata* as the most promising species, which was selected for a deeper chemical investigation. It was possible to determine the differences in the metabolic profiles of stems and leaves, and of collections performed in different states in the Cerrado biome (Minas Gerais and São Paulo). A bio-guided fractionation led to the isolation of nitropropanoyl glucosides, an unusual class of metabolites that showed moderate to potent larvicidal activity. In conclusion, this study contributed to investigate the chemistry of dozens of Malpighiaceae species which had not been investigated to date. It allowed the inspection of the chemical classes in Malpighiaceae in a chemoevolutionary context, besides leading to the isolation of unusual natural compounds by a metabolomic-based approach. These results contributed to expand the chemical knowledge of Malpighiaceae, and the workflows followed can be reproduced in other taxa for further chemical investigations.

Keywords: Quality by Design; chemotaxonomy; plant metabolomics; dereplication; mass spectrometry; evolution; nitro compounds.

RESUMO EXPANDIDO

Os produtos naturais oriundos de plantas, microorganismos e organismos marinhos têm fascinado e inspirado a humanidade desde a antiguidade, principalmente devido aos efeitos terapêuticos observados para os mais diversos tipos estruturais. Estima-se que aproximadamente 50% dos fármacos comercializados são produtos naturais e seus derivados, ou inspirados em produtos naturais (NEWMAN; CRAGG, 2020). De fato, a busca por moléculas inéditas de origem natural foi foco de inúmeros grupos de pesquisa nas últimas décadas, e estima-se que aproximadamente 300.000 metabólitos secundários foram identificados até os dias de hoje, sendo que muitas destas substâncias foram avaliadas para somente um, ou mesmo nenhum alvo biológico (HUBERT; NUZILLARD; RENAULT, 2017). Apesar de centenas de milhares de substâncias naturais terem sido descritas, estima-se que somente uma pequena porcentagem da biodiversidade mundial tenha sido explorada quimicamente. Dessa forma, a área da química de produtos naturais ainda tem muito a ser explorada, principalmente em países considerados *hotspots* de biodiversidade, como o Brasil.

Nas últimas décadas, o desenvolvimento de técnicas hifenadas sensíveis tornou possível estudos mais abrangentes de extratos. Nesse contexto, as técnicas hifenadas ganham destaque, principalmente a cromatografia gasosa e cromatografia líquida acopladas a espectrômetro de massas (CG-EM e CL-EM). A possibilidade de se acoplar um detector extremamente sensível, como o espectrômetro de massas, a uma técnica de separação cromatográfica prévia possibilita explorar amostras complexas (como extratos) de forma mais global. Além disso, o desenvolvimento de bases de dados e ferramentas computacionais de processamento de dados e de anotação, causaram uma revolução na área de produtos naturais.

A fitoquímica clássica com foco de isolar substâncias bioativas foi muito utilizada nas últimas décadas, mas muitas vezes levava ao re-isolamento de substâncias já bastante exploradas. Com o uso das técnicas descritas acima, é possível determinar as substâncias conhecidas a partir de comparação com bases de dados espectrais e focar o estudo em substâncias potencialmente inéditas, ou mesmo em metabólitos conhecidos de interesse. Essa forma mais racional de busca por substâncias naturais tem sido empregada com sucesso na química de produtos naturais, e ferramentas computacionais ainda mais robustas vem sendo desenvolvidas para auxiliar no

processo de interpretação de grandes conjuntos de dados. Esses avanços permitem a realização de trabalhos cada vez mais abrangentes, com quantidades de amostras cada vez maiores.

A família botânica Malpighiaceae se destaca pela diversidade de produtos naturais descritos na literatura. Diversos estudos em Malpighiaceae dividem essa família em dez grandes grupos filogenéticos, compreendendo 74 gêneros e cerca de 1300 espécies distribuídas nas regiões tropicais e subtropicais do globo (DAVIS; ANDERSON, 2010). Estudos químicos prévios demonstraram que as espécies de Malpighiaceae acumulam uma ampla gama de metabólitos secundários, incluindo alcaloides β-carbonílicos e derivados de triptamina, isoprenoides (como fitoecdisteroides), e compostos fenólicos abrangendo diferentes subclasses de flavonoides a derivados de ácido quínico (FRAIGE *et al.*, 2018; MANNOCHIO RUSSO *et al.*, 2020; QUEIROZ *et al.*, 2014; SAMOYLENKO *et al.*, 2010). Além da complexidade química, as espécies de Malpighiaceae também se destacam pelo valor nutricional de seus frutos, usos etnofarmacológicos, e propriedades tóxicas. Apesar de diversos estudos químicos reportados, essa família ainda é considerada quimicamente pouco explorada, com centenas de espécies que ainda não foram estudadas com esse propósito. Dessa forma, um estudo químico abrangente em Malpighiaceae é de grande interesse para ampliar o conhecimento químico da família, e correlações quimiotaxonômicas (i.e., classes de substâncias específicas ou mais abundantes em determinados clados filogenéticos ou gêneros) podem auxiliar a guiar futuros estudos nessa família.

A produção de compostos tóxicos por espécies de Malpighiaceae despertam interesse na busca de substâncias com as mais diversas bioatividades. Um estudo prévio realizado com extratos de *Hiptage benghalensis* revelou que essa espécie apresentava importantes atividades inseticidas e repelentes contra mosquitos transmissores de doenças negligenciadas em países do sudeste asiático (LALROTLUANGA *et al.*, 2012). Dessa forma, essa família botânica pode ser promissora para a busca de substâncias tóxicas para o combate de mosquitos transmissores de doenças negligenciadas, como o *Aedes aegypti*. Esse mosquito é o vetor de diversas doenças que se espalham em território nacional todos os anos, como a dengue, zika e chikungunya. Vacinas e tratamento efetivos para essas doenças ainda não foram desenvolvidos, e a forma mais efetiva de combate a essas doenças é o controlar o vetor (SILVÉRIO *et al.*, 2020).

Dessa forma, o presente trabalho tem como principais objetivos realizar uma ampla investigação química de 137 espécies de Malpighiaceae, visando utilizar ferramentas computacionais recentemente descritas para encontrar correlações quimiossistemáticas e buscar compostos bioativos. Nesse contexto, dividimos esta tese em três capítulos para melhor compreensão de seu conteúdo:

1. Desenvolver um método cromatográfico UHPLC de uma mistura de extratos de Malpighiaceae usando a abordagem Quality by Design e automação instrumental, e anotar os metabólitos secundários com base no algoritmo de rede molecular e comparação com bibliotecas espectrais MS/MS.
2. Realizar uma investigação quimiotaxonômica usando metabolômica não direcionada, redes moleculares, buscas em bibliotecas espectrais e ferramentas *in silico* para classificação de metabólitos para correlacionar os resultados químicos com a filogenia atualmente aceita para a família.
3. Buscar por compostos com atividade larvicida contra o *Aedes aegypti* através de uma triagem inicial de extratos de Malpighiaceae, seguida de uma investigação química mais aprofundada da espécie mais ativa por meio de uma abordagem metabolômica.

O primeiro capítulo dessa tese descreve uma metodologia analítica para desenvolver um método cromatográfico robusto para análise de espécies de Malpighiaceae, além da anotação dos metabólitos secundários produzidos por estas. Para tal, uma mistura (MIX) em partes iguais de nove extratos hidroetanólicos de Malpighiaceae foi preparada, compreendendo uma espécie de cada grupo filogenético da família, sendo estas: *Byrsonima intermedia*, *Mcvaughia bahiana*, *Barnebya harleyi*, *Ptilochaeta densiflora*, *Bunchosia pallescens*, *Hiraea restingae*, *Niedenzuella multiglandulosa*, *Banisteriopsis laevifolia* e *Amorimia septentrionalis*. Os extratos de cada uma dessas espécies foram obtidos por micro-extração em ultrassom. O MIX foi, portanto, utilizado para desenvolvimento do método cromatográfico utilizando a abordagem de Quality by Design (QbD). Para tal, contamos com o auxílio do software Fusion Quality by Design® e de automação instrumental para podermos avaliar diversos parâmetros em um curto tempo.

O desenvolvimento do método em *Ultra-High Performance Liquid Chromatography- photodiode array* (UHPLC-PDA) contou com três principais etapas: triagem, otimização e simulação de robustez. Além disso, as respostas selecionadas

para análise estatística foram: número de picos totais, número de pares de picos com resolução ≥ 1.5, número de pares de picos com resolução ≥ 2.0, e número de picos com encaudamento ≤ 1.2. Na primeira etapa foram avaliados seis colunas, dois solventes orgânicos, três valores de pH (ácido, neutro e básico) e dois tempos de gradiente. Na segunda etapa, a temperatura, pH (três valores de acidez), % CH_3CN–MeOH em fase orgânica e % final de solvente orgânico foram variados. A etapa final consistiu no simulador de robustez, que proporcionou as melhores condições: coluna Acquity BEH Shield RP18 (2.1 x 100 mm, 1.7 µm), CH_3CN–MeOH (80/20):H_2O, pH 2.22, 275 nm, 45 °C, 5-55% B em 30 min. Devido à automação computacional e instrumental, foi possível chegar a esses resultados em apenas quatro dias.

Esse trabalho também incluiu a validação do método desenvolvido através do uso dos padrões (+)-catequina, (−)-epicatequina, rutina, e ecdisterona, baseado nas diretrizes do ICH (*International Conference on Harmonization*). O método atingiu todos os critérios estabelecidos, provando ser extremamente sensível até mesmo para diversas classes de substâncias. Além disso, a análise dos nove extratos individualmente também mostrou uma separação satisfatória dos picos de cada extrato, indicando que o uso de uma mistura de extratos para o desenvolvimento do método foi uma abordagem eficaz.

Finalmente, esses extratos individuais foram analisados por UHPLC-MS/MS em um método não direcionado. O uso da plataforma *Global Natural Product Social Molecular Networking* (GNPS) possibilitou a obtenção de redes moleculares e comparação com bibliotecas espectrais públicas. Essa abordagem levou à anotação de 61 compostos, incluindo flavonoides *O*-glicosilados, flavonoides *C*-glicosilados, derivados de ácido quínico, esteróis, e outros compostos fenólicos.

Dessa forma, os resultados desse capítulo mostraram a eficácia de se empregar uma mistura de extratos, do uso de automação e design experimental na obtenção de métodos cromatográficos sensíveis, e da utilização da plataforma GNPS como uma ferramenta importante para anotação de substâncias conhecidas em extratos. Esse trabalho pode servir de inspiração para acelerar o fluxo de trabalho em pesquisas na área de produtos naturais, uma vez que tempo e recursos são poupados. Além disso, foi possível ter as primeiras informações químicas acerca de três gêneros de Malpighiaceae até então ainda não explorados quimicamente: *Mcvaughia*, *Barnebya* e *Ptilochaeta*.

O segundo capítulo da tese teve como principal foco o estudo quimiossistemático da família Malpighiaceae. Para tal, uma amostragem compreendendo 39 gêneros e 137 espécies foi empregada, compreendendo todos os dez grandes clados filogenéticos da família. Extratos hidroetanólicos (80%) e acetato de etila foram preparados a partir de 197 amostras (em sua grande maioria, folhas) por micro extração em ultrassom. Essas amostras foram, portanto, analisadas por UHPLC-MS/MS em um método não directionado, tanto no modo positivo quanto negativo de ionização. Dessa forma, um total de 788 corridas foram realizadas. Os dados foram processados no software MZmine2 para análises subsequentes.

Inicialmente, uma avaliação do impacto do solvente extrator e modo de ionização foi realizada a partir de Análise de Coordenadas Principais (PCoA). A partir desses resultados, foi possível concluir que tanto o solvente extrator quanto o modo de ionização impactam significativamente o perfil químico dos extratos. Além disso, correlacionando os grupos gerados nessas análises multivariadas com a filogenia atualmente aceita para a família, foi possível verificar que, diferentemente dos extratos hidroetanólicos (80%), os grupos observados para os extratos acetato de etila não mostraram correlação com a filogenia. Dessa forma, foi possível inferir que os protocolos de extração podem impactar significativamente em estudos quimiossistemáticos.

A partir dos extratos hidroetanólicos (80%) adquiridos tanto em modo positivo quanto negativo, foram geradas redes moleculares e os espectros MS/MS foram comparados com as bibliotecas espectrais públicas da plataforma GNPS. Diversas famílias mostraram-se específicas de determinados clados filogenéticos. Dentre as principais famílias moleculares consideradas específicas de clados, destacam-se: flavonoides di-*C*-glicosilados (clado I), ácido quínico ligados a substituintes galloil (clados A e G), ácido quínico ligados a substituintes fenilpropanoil (clado H), alcaloides benzilisoquinolínicos (clado J), ecdisteroides (clado H) e iridoides (clado J).

De modo a expandir o conhecimento químico da família, e ter informações adicionais sobre as classes de compostos detectadas, utilizamos o *plug in* Qemistree combinado à ferramenta CANOPUS de classificação *in silico*. Dessa forma, foi possível realizar uma análise de hierarquia química e obter classificação *in silico* a nível de superclasse, classe e subclasse. Os resultados obtidos em modo positivo de ionização foram consideravelmente diferentes e complementares ao modo negativo

de ionização, principalmente pois as bases espectrais são mais populadas com dados em modo positivo.

Os resultados das anotações *in silico* foram utilizados para realizar análises de reconstrução de caráter ancestral, com o intuito de mapear a história evolutiva dessas classes usando os critérios de máxima verossimilhança na filogenia molecular de Malpighiaceae. A partir dessa análise, os dados das classificações obtidas em modo positivo e negativo foram combinados, e as classificações foram recuperadas como homoplasias (não-exclusivas) e sinapomorfias (exclusivas) dos principais clados filogenéticos da família.

Dessa forma, os resultados desse capítulo mostram uma nova abordagem para estudos quimiossistemáticos abrangentes utilizando a família Malpighiaceae como modelo. A partir de pouca quantidade de material vegetal e de análises extremamente sensíveis, foi possível obter uma grande quantidade de informações químicas a partir de comparação com bibliotecas espectrais públicas e uso de ferramentas *in silico* de classificação. A comparação dessas classes com a filogenia possibilitou a interpretação desses resultados em um contexto evolutivo da família, sendo extremamente relevante para estudos quimiotaxonômicos. Esses resultados devem ser confirmados em futuros estudos mais aprofundados em cada espécie ou gênero, e para tal, as ferramentas clássicas são de grande valia. A partir desse trabalho, dezenas de espécies de Malpighiaceae sem qualquer estudo químico prévio foram exploradas pela primeira vez, iluminando o metabolismo desse táxon ainda pouco estudado. O fluxo de trabalho seguido nesse capítulo utilizou ferramentas e bases de dados publicamente disponíveis, e pode ser seguido em estudos em outros táxons pouco explorados.

O terceiro capítulo dessa tese consistiu na busca de substâncias naturais com atividade larvicida contra o *Aedes aegypti* em Malpighiaceae, utilizando uma abordagem metabolômica. A mesma coleção de amostras utilizada no segundo capítulo foi empregada nesse trabalho para se fazer uma triagem inicial. Dessa forma, extratos hidroetanólicos (80%) e acetato de etila obtidos por micro-extração em ultrassom foram avaliados em ensaios larvicidas contra *Ae. aegypti*. Essa triagem inicial mostrou que as espécies *Tetrapterys phlomoides* e *Verrucularina piresii* apresentaram 70% e 65% de mortalidade após 72 h de incubação, enquanto a *Heteropterys umbellata* causou 100% de mortalidade após apenas 24 h. Nenhuma dessas espécies apresenta relatos de estudos químicos e biológicos aprofundados

até o momento. Dessa forma, maiores quantidades de materiais vegetais de *H. umbellata* foram coletadas para dois órgãos vegetais diferentes (folhas e caule) em dois estados diferentes (São Paulo e Minas Gerais), totalizando 14 amostras para investigação metabolômica.

As amostras coletadas foram submetidas a extração por maceração em solução hidroalcoólica (80%), e os extratos obtidos foram analisados por HPLC-MS/MS em um método não direcionado, em triplicata. Os dados foram processados no software MZmine2 e submetidos a análises nas plataformas MetaboAnalyst (para análise multivariada) e GNPS (para obtenção das redes moleculares e comparação com as bibliotecas espectrais). Na plataforma MetaboAnalyst, os dados foram submetidos a análise não supervisionada (*Principal Component Analysis*, PCA) e supervisionada (*Partial Least Squares Discriminant Analysis*, PLS-DA). Essas análises revelaram que os perfis químicos obtidos dos extratos das folhas são significativamente diferentes dos extratos do caule. Além disso, o estado de coleta também tem um impacto significativo no perfil metabólico quando somente os extratos de folhas ou de caules são considerados. Através da análise de PLS-DA também foi possível recuperar uma lista das substâncias que impactam mais significativamente da diferenciação dos grupos descritos. Dessa forma, foi possível direcionar a anotação dessas substâncias de maior interesse.

Os resultados das redes moleculares e busca nas bibliotecas espectrais publicamente disponíveis no GNPS mostraram três principais famílias moleculares com correspondências: flavonoides *O*-glicosilados, flavonoides *C*-glicosilados, e derivados de derivados de ácido cafeoilquínico. Essas duas últimas classes compreendiam substâncias relevantes recuperadas nas análises de PLS-DA para a diferenciação de órgãos da planta e estado de coleta. Além disso, outras duas substâncias abundantes não mostram correspondência alguma com as bibliotecas, sendo recuperadas como importantes na diferenciação dos pontos de coleta – tanto para folhas, quanto para caule. Essas substâncias poderiam ser substâncias potencialmente inéditas (ou incomuns), e o fato de serem menos polares também chamou atenção devido ao potencial da atividade buscada.

Portanto, esforços foram direcionados para o isolamento dessas substâncias, que foram caracterizadas por Espectrometria de Massas de Alta Resolução, Ressonância Magnética Nuclear, Espectrometria de Massas em Tandem, sendo caracterizadas como 1,2,6-tris-*O*-[3-nitropropanoyl]-beta-D-glucopyranose (karakin, **2**) e 1,2,3,6-

tetrakis-*O*-[3-nitropropanoyl]-beta-D-glucopyranose (**3**). A substância 3,5-di-*O*-caffeoylquinic acid (isochlorogenic acid A, **1**) também foi isolada por se tratar de uma substância recuperada como relevante em todas as análises de PLS-DA realizadas. Os nitrocompostos isolados são considerados incomuns na natureza, com poucos relatos em poucas famílias botânicas e já relatados para outras espécies de *Heteropterys*.

Essas substâncias isoladas foram subsequentemente utilizadas para realizar a validação do método de análise, utilizando um método direcionado para recuperar as principais transições MS/MS no modo *Multiple Reaction Monitoring* (MRM). O método se mostrou sensível para os compostos em questão, e consiste no primeiro método validado que permite a quantificação desses ésteres intactos. Essas três substâncias foram quantificadas nos 14 extratos inicialmente obtidos, e foi possível confirmar os resultados obtidos para as análises de PLS-DA, além de levantar hipóteses de possíveis funções ecológicas. Os compostos nitrogenados mostraram moderada atividade larvicida quando testados puros, mas a atividade foi potencializada quando foram testados em fração contendo outros isômeros com mesmo padrão de fragmentação, sugerindo um possível efeito sinergístico na atividade.

Os resultados do terceiro capítulo da tese demonstram que a família Malpighiaceae representa um táxon promissor para a busca de compostos larvicida para o combate de vetores de arboviroses. Através do uso de abordagens metabolômicas, foi possível realizar um estudo aprofundado da espécie mais ativa selecionada da triagem inicial. Essas ferramentas levaram à seleção de compostos nitrogenados incomuns na natureza e que ainda não haviam sido avaliadas para a bioatividade em questão. Esperamos que esse estudo possa guiar futuras pesquisas com o gênero *Heteropterys*, além de inspirar outras investigações na área da química de produtos naturais utilizando ferramentas metabolômicas.

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LIST OF ACRONYMS

®	Registered trademark
*g*COSY	Correlation Spectroscopy
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
*g*DEPTQ	Distortionless Enhancement by Polarization Transfer with quaternary carbon detection
ESI	Electrospray Ionization
FBMN	Feature-Based Molecular Networking
GNPS	Global Natural Product Social Molecular Networking
*g*HMBC	Heteronuclear Multiple Bond Coherence
HPLC	High Performance Liquid Chromatography
HPLC-PDA	High Performance Liquid Chromatography - Photodiode Array Detector
HRMS	High-Resolution Mass Spectrometry
*g*HSQC	Heteronuclear Single Quantum Coherence
J	Coupling constant
LC-MS	Liquid Chromatography - Mass spectrometry
m	multiplet
m/z	Mass to charge ratio
MS	Mass Spectrometry
MS/MS	Tandem mass spectrometry
NMR	Nuclear Magnetic Resonance
*g*NOESY	Nuclear Overhauser Effect Spectroscopy
PCA	Principal Component Analysis
PLS-DA	Partial Least-Squares Discriminant Analysis
QToF	Quadrupole time-of-flight
Rt	Retention time
s	singlet
t	triplet
UHPLC	Ultra-High-Performance Liquid Chromatography

VIP score	Variable in Importance in Projection score
δ	Chemical shift
δC	Carbon chemical shift
δH	Hydrogen chemical shift

SUMMARY

1 INTRODUCTION

The chemistry of natural products from plants, microorganisms, and marine organisms has fascinated and inspired humanity since ancient times, mainly due to the therapeutic effects reported for the most diverse structural types, being the molecular base of several drugs used in the treatment and prevention of diseases. These compounds of natural sources are biosynthesized through complex pathways, originating a significant number of pharmacophoric groups with high selectivity, being a rich source for the production of drugs, chemical pesticides, fragrances, cosmetics, food supplements, among others. The impressive number of compounds from natural origin identified to date are important sources to identify hits and leads, even for the most challenging targets (HARVEY; EDRADA-EBEL; QUINN, 2015).

For many decades, the search for novel molecules of natural origin has been the focus of numerous research groups on all continents, and it is estimated that approximately 300,000 secondary metabolites have been identified to date, with many of these substances being evaluated for only one, or even none, biological target (HUBERT; NUZILLARD; RENAULT, 2017). Traditionally, the active principles in a bioactive extract are identified using purification strategies, such as liquid-liquid extraction and chromatographic separation methods. This approach demands high effort and investment, and can lead to the isolation of well-known substances, or even without the expected activity (NOTHIAS *et al.*, 2018).

Considering the large number of known natural compounds, new strategies have been employed to save time, cost, and effort spent on research in the chemistry of natural products field. The development of increasingly sensitive hyphenated analytical techniques, such as GC-MS, HPLC-MS, HPLC-NMR, HPLC-NMR-MS, and HPLC-SPE-NMR, enables researchers to perform broader scope chemical investigations using metabolomic approaches.

1.1 Metabolomics in natural products investigations

Metabolomic studies allow a more comprehensive overview of the metabolites (usually in the range of 50 to 1500 Da) produced by a specific organism. Consequently, one can investigate if, and how, groups chemically differ as a response to biotic stimuli,

stress, environmental changes, among others. Basically, these studies can be classified as targeted or untargeted, in which the first one will focus on possible chemical changes in pre-selected compounds, while the second one will look at the whole data aiming to annotate and/or quantify as many compounds as possible (PILON *et al*., 2020).

Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) are among the most widely used hyphenated techniques in metabolomic studies for their great sensitivity, and for the previous chromatographic separation. In fact, LC-MS has shown great acceptance by the scientific community due to the possibility of detecting a larger number of intact metabolites without the need for chemical derivatization (GOWDA; DJUKOVIC, 2014).

In metabolomic studies, several statistical analyses can be used to investigate a given dataset and how groups can chemically differentiate. For instance, supervised multivariate analyses, such as partial least squares discriminant analysis (PLS-DA), or non-supervised multivariate analyses, such as Principal Component Analysis (PCA), are widely employed. On the other hand, the univariate analyses are also largely used to evaluate the variables individually, and the relationships between them are not considered; in this case, statistical tests such as Analysis of Variance (ANOVA) and Student's t-test are commonly employed (PILON *et al*., 2020).

Besides these chemometric approaches, the annotation of known compounds (also called "dereplication") is of great interest for a biological interpretation of the hypothesis initially raised. In the case of LC-MS, the annotation of these compounds can be performed by comparing the experimental mass spectra with the ones reported in the literature. By identifying the known compounds in a complex mixture, it is possible to focus the research on potentially new compounds, or even isolate known substances for a biological reassessment (GAUDÊNCIO; PEREIRA, 2015; HUBERT; NUZILLARD; RENAULT, 2017).

Even though there has been an evident development of analytical techniques and chemometrics analyses, the annotation of compounds in complex matrices is still considered time-consuming when performed manually. This is particularly critical for large datasets, and nuances can be lost in manual comparisons between analyses.

It is estimated that, considering tandem mass spectra (MS/MS) data obtained from a biological sample, only about 1.8% of the spectra of an untargeted analysis can be determined by comparison with existing data (QUINN *et al*., 2017). With such

approaches, 98% of the spectra obtained in these analyses can be called the “dark matter” of metabolomics (SILVA; DORRESTEIN; QUINN, 2015). It consists of a vast amount of chemical information that, at first, does not help in identifying known compounds.

In recent years, increasingly sophisticated computational methods have been developed to aid and accelerate data processing and annotation. In this context, the molecular networking is an approach that has been successfully used in the natural products field, becoming a powerful and valuable computational strategy for the visualization and interpretation of MS/MS data acquired.

1.2 Molecular networking and the GNPS platform

The molecular networking approach has been widely used in recent studies on the chemistry of natural products. It has proven to be a valuable computational strategy for interpreting mass spectrometry data obtained from complex matrices. The use of molecular networking for the study of natural products led to the development of an open-access platform called GNPS (Global Natural Product Social Molecular Networking), in which it is possible to store, analyze and share information about MS/MS data. In addition, several reference spectral libraries are publicly available on the platform, which is a valuable asset to assist in annotating known compounds in a complex mixture or dataset (QUINN *et al*., 2017; WANG *et al*., 2016; YANG *et al*., 2013). On this platform, MS/MS data can be analyzed and, after similarity calculations of the fragmentation patterns of the substances present in the samples, it is possible to generate a map consisting of molecular families (or networks). These networks are made up of nodes and edges, where a node corresponds to an MS/MS spectrum of a given substance, while the edges connecting these nodes indicate the degree of similarity between the spectra by cosine similarity. In other words, the connection between the nodes represents structurally similar molecules sharing similar MS/MS fragmentation patterns. An illustrative scheme of this approach is shown in Figure 1.

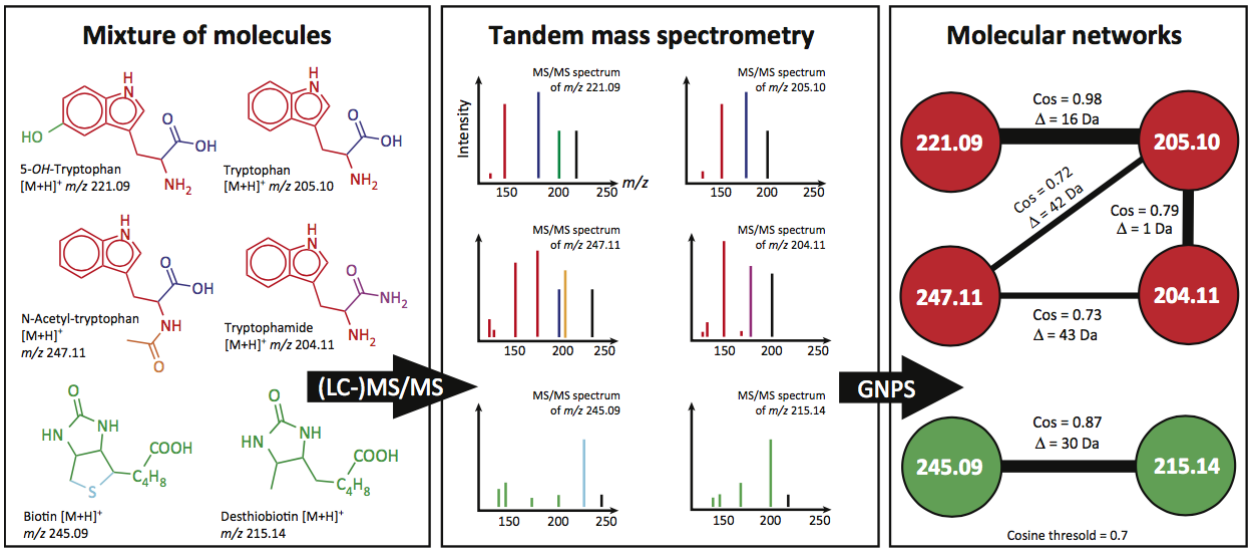

Figure 1 – Illustrative scheme of the Molecular Networking approach: a mixture of compounds is analyzed by LC-MS/MS and then organized into molecular families through computational methods.

Source: Quinn *et al.* (2017).

When a large number of samples is analyzed, the results obtained using the Molecular Networking approach make it possible to find correlations between classes of compounds with the presence or absence of genes that encode them, being particularly useful in studies regarding biosynthetic pathways and their evolution (QUINN *et al.*, 2017). The Molecular Networking approach has been widely used in articles published in literally thousands of high-impact indexed journals in recent years. Particularly in the natural products field, for instance, this tool can be applied to 1) reinvestigate a well-known species in order to identify new natural compounds (FOX RAMOS *et al.*, 2017); 2) study bioactive compounds by combining bioactivity data from natural products and MS/MS spectra (NOTHIAS *et al.*, 2018); 3) study libraries of extracts from a specific taxon (OLIVON *et al.*, 2018).

An article with this later focus consisted in investigating 292 extracts obtained from 107 species of the Euphorbiaceae family collected on New Caledonia. These extracts were analyzed by UHPLC-MS/MS, and the analytical data obtained were subjected to the Molecular Networking approach in the GNPS platform. In this case, the nodes were colored based on the genera each feature was detected genera, and genera-specific networks were obtained (OLIVON *et al.*, 2018), as depicted in Figure 2.

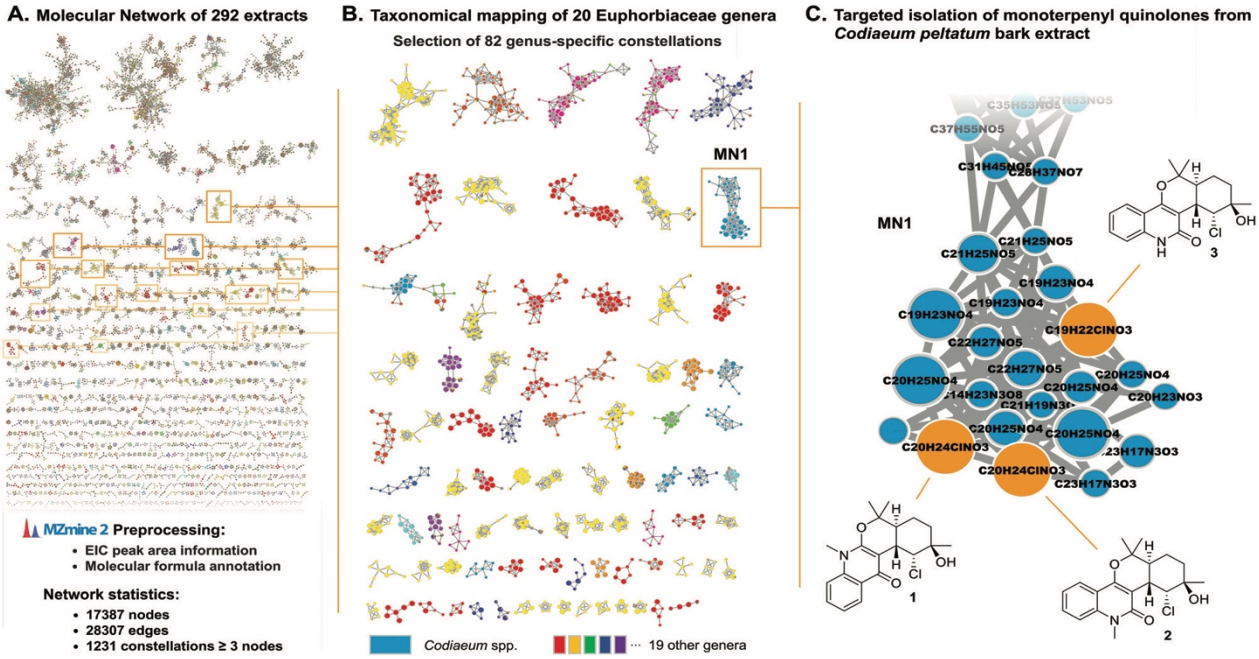

Figure 2 – (**A**) Molecular networks obtained for the 292 extracts of Euphorbiaceae species, in which a different color was assigned to each genus; (**B**) Networks characterized by only one color, indicating specific chemical structures of certain genera; (**C**) Selection of chlorinated substances of interest and targeted isolation of these substances in *C. peltatum*. Node size is proportional to the MS^1 peak area of the crude extract.

Source: Olivon *et al*. (2018).

These results show the importance of state-of-the-art computational tools in the chemistry of natural products from a more comprehensive point of view. It provides valuable support for the metabolomic studies of complex matrices and assists phylogeny studies of poorly studied taxa. In addition, the molecular networking approach combined with searches in spectral libraries also enables the direct search for potential new natural compounds with promising bioactivities. However, despite the evident boost in the annotation of known compounds due to the growing number of spectral databases, this step is still considered a bottleneck in the natural products field. Within this scenario, other computational tools can be used to assist in the annotation process.

1.3 In silico tools for metabolites annotation

From the estimated 2% average annotation rate estimated in 2017 for complex samples (QUINN *et al.*, 2017), recently published articles have described annotation up to more than 10% depending on the sample type (DEAN *et al.*, 2021). Part of this jump is a consequence of the scientific community's effort to populate public spectral libraries with experimental spectra of known compounds. However, there is still much to be chemically explored, and several *in silico* tools have been developed in the last decade to expand the annotation of chemical compounds in MS/MS datasets (BAUERMEISTER *et al*., 2022; MEDEMA, 2021; MISRA, 2021).

These *in silico* tools make use of structural databases and enable users to have more general information regarding the classes of the compounds detected. The information retrieved from such tools has lower confidence than the spectral library searches, and is relative to a level 3 according to the Metabolomics Standards Initiative (MSI) (SUMNER *et al*., 2007). Even though there is a lower confidence level in the*in silico* annotations, they are extremely useful for gathering broad chemical information and expanding chemical knowledge of large datasets with a very low annotation rate retrieved from spectral searches.

There are dozens of these tools reported in the literature making use of sophisticated algorithms to predict the chemical classes through the most diverse methods. For instance, SIRIUS uses a method based on the computation of fragmentation trees to select the molecular formula that best explains the MS data of a particular compound and, along with CSI:FingerID, it predicts a molecular fingerprint encoding substructural properties of the molecule (DÜHRKOP *et al*., 2015, 2019). On the other hand, MetFrag uses combinatorial fragmentation methods to explain MS/MS peaks based on substructures generated by disconnecting the bonds of known chemical compounds (WOLF *et al*., 2010).

In fact, *in silico* classification tools have also been combined with molecular networking, such as the Network Annotation Propagation (NAP). NAP is based on the MetFrag algorithm, and it makes it possible to compare the structural candidates of each node in a network and re-rank a particular structure when a connected node has a related structure in the network (SILVA *et al.*, 2018).

A few studies in the literature make use of specific *in silico* classifications tools in chemotaxonomy investigations, and it has proven to be a promising approach to explore large plant datasets in an evolutionary context (KANG *et al*., 2019). In this way,

the expanded chemical knowledge retrieved from such tools can be of great value to explore plant chemotaxonomy at a family level.

1.4 Malpighiaceae botanical family

The Malpighiaceae family belongs to the group of angiosperms and presents a great diversity of forms such as trees, shrubs, and vines, found in tropical, subtropical, and savannah regions. This family currently consists of 74 genera and approximately 1300 species (http://powo.science.kew.org/), located in the New and Old World. It is estimated that about 85% of plant species belong to the New World, while the other 15% are found in other tropical and subtropical regions of the globe. Furthermore, approximately 150 species belonging to 17 genera are found exclusively in the Old World (DAVIS *et al.*, 2002; DAVIS; ANDERSON, 2010).

Most Malpighiaceae species occur in South America, north of the Tropic of Capricorn, with Brazil being the South American country with the highest representation of this family (ANDERSON, 2004). Approximately 46 genera and 588 species are found in the entire national territory, predominantly in the Atlantic Forest, Cerrado, and Amazon biomes (ALMEIDA *et al.*, 2021).

The largest genera of this family are *Heteropterys* (151 species), *Byrsonima* (136), *Stigmaphyllon* (117), *Bunchosia* (79), *Hiraea* (74), *Banisteriopsis* (59), *Malpighia* (53), and *Tetrapterys* (50), and 16 genera are made up of only one species (http://powo.science.kew.org/). It should be noted that several taxonomic revisions of the species in this family have been described, and a species previously belonging to a genus can be reclassified to a different (or new) genus. For example, some *Tetrapterys* species (such as *T. multiglandulosa* and *T. acutifolia*) were reclassified to the genus *Niedenzuella* (*N. multiglandulosa* and *N. acutifolia*) (ANDERSON, 2006), while *T. cardiophylla* and *T. microphylla* were reclassified to the *Glicophyllum* genus (*G. cardiophyllum* and *G. microphyllum*) (ALMEIDA; VAN DEN BERG, 2021).

Byrsonima crassifolia and *Malpighia emarginata* are two of the economically important species of Malpighiaceae, whose fruits are popularly known as murici and acerola, respectively, and are widely consumed in Brazil. In addition to the nutritional properties, several species of this family are also ornamental, such as *Stygmaphyllon floribundum*, *Lophanthera lactescens*, and *Malpighia coccigera* (ANDERSON, 2004).

One of the most studied Malpighiaceae species is *Banisteriopsis caapi*, whose vine has been known since pre-Columbian times and used in the preparation of Ayahuasca tea. This tea is consumed to this day, not only in indigenous rituals but also in syncretic religious movements in Brazil, such as the União do Vegetal and Santo Daime. It is prepared by the decoction of the vine of *B. caapi* and the leaves of *Psychotria viridis* (Rubiaceae), and the substances present in this tea affect the central nervous system when ingested due to the toxic components and hallucinogens present (MCKENNA; CALLAWAY; GROB, 1998). Phytochemical studies carried out with *B. caapi* showed that this species accumulates, mainly in its vine, β-carboline alkaloids, which are generally very toxic (SAMOYLENKO *et al.*, 2010).

Another species of Malpighiaceae, morphologically very similar to *B. caapi*, is *Tetrapterys mucronata*. Due to the morphological similarity, *T. mucronata* can be erroneously used instead of *B. caapi* to prepare this tea. In studies carried out recently by the NuBBE research group, the presence of β-carboline alkaloids similar to the ones described for *B. caapi* was determined in *T. mucronata*. However, these are even more toxic than the ones present in *B. caapi* and can lead to death when ingested (QUEIROZ *et al.*, 2014, 2015).

In addition to species toxic to humans, outbreaks of acute and chronic cattle poisoning have also been reported in central Brazilian regions caused by species belonging to *Amorimia* (LEE *et al*., 2012) and *Niedenzuella* (mainly *N. multiglandulosa* and *N. acutifolia*) genera (CARVALHO *et al*., 2008; RIET-CORREA; MEDEIROS; SCHILD, 2012). Since these species are considered invasive of pasture fields, these plants can cause considerable economic losses since the Brazilian economy is highly dependent on agribusiness. In this way, livestock producers are committed to eliminating these species from pasture fields, and some of these species (mainly *Amorimia*) are becoming more difficult to find in nature. Therefore, these species should be studied for a better chemotaxonomic understanding of the family and the economic problems caused. With this concern, a recent phytochemical study for *Niedenzuella multiglandulosa* was performed in the NuBBE research group, in which several phytoecdysteroids were identified in the leaves of this species (MANNOCHIO RUSSO *et al.*, 2020).

Several other species of this family are reported to present biological activities, such as antifungal (*Heteropterys byrsonimifolia*), antidepressant, and anxiolytic (*Heteropterys brachiata*) (HUERTA-REYES; FONSECA JUÁREZ; AGUILAR-ROJAS,

2015). However, many species and genera still do not present any chemical study. Therefore, a more in-depth study of this family is of interest for a better chemical understanding of this taxon.

1.5 Chemosystematics studies of plants

Plant systematics can be defined as the science of the diversification of an organism, involving the discovery, description, and interpretation of the biological diversity existing on the planet today, considering its evolutionary history. For this, it is necessary to study all branches of an evolutionary tree in order to document the changes that occurred during the evolution of these branches, and to describe all species (the tips' branches) (JUDD *et al*., 2002; STUESSY, 2008).

There are several manners to carry out the taxonomic classification of living organisms. Particularly for plants, the approach can be carried out based on their most suitable habitat, observing the macromorphology of the species, among others. The macromorphological classification was one of the first to be used to infer evolutionary relationships (phylogeny) (JUDD *et al*., 2002). It is also worth mentioning that evolution depends on a series of internal and external factors such as mutation, recombination of genetic differences, and selection.

From the angiosperm evolution point of view, a generation of morphologically similar plants can occur in the evolutionary process without having a common ancestor. This phenomenon, known in biology studies, is called "convergence" or "parallel development". On the other hand, evolutionarily related plants can be morphologically different (in this case, the phenomenon is called "divergence") (ERDTMAN, 1963). Considering these phenomena, the taxonomic classification may, in some cases, be imprecise. Recent taxonomic studies use molecular tools (a more precise and robust approach) in taxonomic studies regarding the reclassification of many previously misclassified species (ANDERSON, 2006). In fact, molecular approaches in consonance with chemical data can assist taxonomic classification of species and assist phylogeny studies.

The classification of plants based on their secondary metabolites has emerged as another promising possibility. This classification, called chemosystematics (or chemotaxonomy), is valuable to assist studies in taxonomy and ecology. Chemosystematic studies are, in principle, little dependent on classical botanical

studies. In this way, the results obtained can help in the classification of a species and confirm whether the classifications and conclusions obtained previously (based on the morphological characters, for instance) also make sense from the point of view of chemical diversity (ERDTMAN, 1963).

In the last decades, most of the chemosystematics studies were conducted based on vast literature surveys of isolated and characterized compounds. Several important conclusions were withdrawn from such studies, correlating geological times with the origin of classes of metabolites and their oxidation levels (GOTTLIEB, 1989). These studies can provide valuable information about the biosynthetic pathways present in the species, which would make it possible to target enzymatic and biotechnological studies, which are very useful for plants (BHARGAVA; PATEL; DESAI, 2013).

Due to the advances in highly sensitive hyphenated techniques, data processing, and low amounts of plant material necessary for such study, comprehensive chemosystematic studies can be carried out through collaborative research between botanists and chemists. In this way, a more comprehensive understanding of the secondary metabolism at a botanical family level can be performed (STUESSY, 2008).

1.6 Systematics of the Malpighiaceae botanical family

Given the interest of Malpighiaceae species for the Brazilian economy, a more detailed study of this family is of interest, including mapping natural products that can help in the taxonomy and phylogeny of the family. Since the 19th century, Malpighiaceae species have been classified into subfamilies and tribes according to the characteristics of their fruits, classified as dry or fleshy fruits. For centuries this classification was accepted and increasingly detailed for differentiating genera into large groups. However, in 2001, the first studies on the molecular phylogeny of Malpighiaceae were published, and a new classification was proposed for the family (CAMERON *et al*., 2001; DAVIS; ANDERSON; DONOGHUE, 2001), based on the plastid *ndhF* , *matK* , and *rbcL* and nuclear *PHYC* (DAVIS; ANDERSON, 2010).

Currently, this family is divided into ten major phylogenetic groups (ALMEIDA *et al.*, 2017; DAVIS; ANDERSON, 2010), and it can be observed that similar morphologies in the fruits originated independently several times during the diversification of the family. In other words, species with fleshy or dry fruits do not have the same common

ancestor. The classification of the ten major phylogenetic groups, along with the distribution of fruit types in the family, is illustrated in Figure 3.

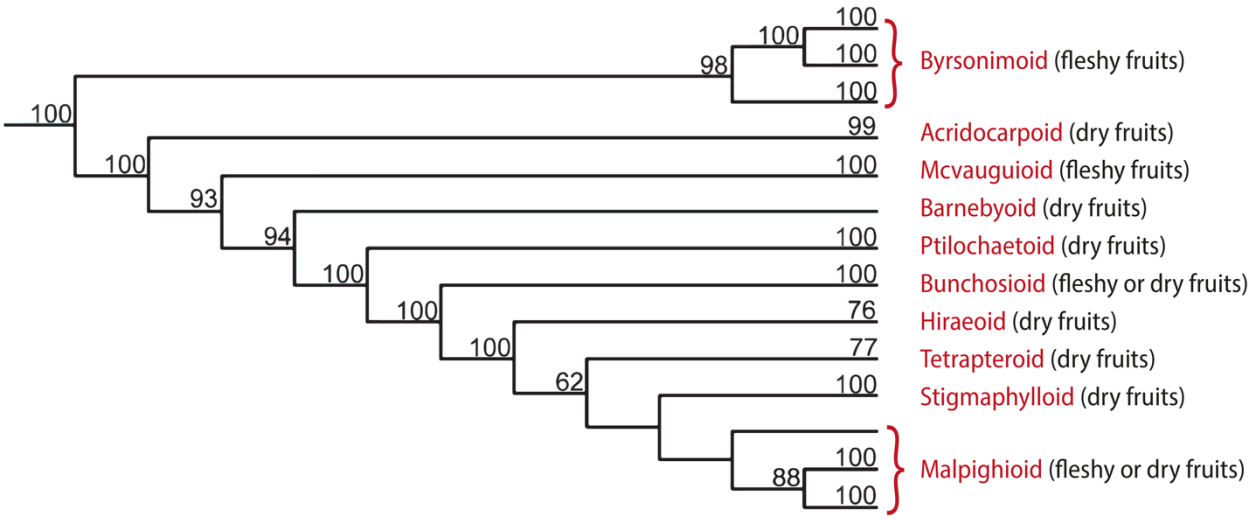

Figure 3 – Major phylogenetic groups (clades) currently accepted for the Malpighiaceae botanical family.

Source: Adapted from Davis and Anderson (2010).

Since these DNA-based studies, several authors have gradually proposed new genera and combinations to accommodate these newly identified genetic lineages, and many species still lack such kind of study. In addition, no morphological characters were ever recovered, circumscribed, or discussed for its major phylogenetic clades (ALMEIDA; VAN DEN BERG, 2021). In this way, chemical studies and metabolomic tools are of great value to assist in establishing a new classification system based on chemical compounds and morphology (ALMEIDA *et al.*, 2017).

1.7 Bioactive natural products as candidates for Aedes aegypti mosquito control

Mosquitoes are vectors of several human diseases (also called arboviruses), such as malaria, dengue, zika, filariasis, and yellow fever, among many others, being more frequent in tropical and subtropical regions of the world (GERIS *et al.*, 2012). The *Aedes aegypti* mosquito (Diptera: Culicidae) is the primary vector of dengue, zika, and chikungunya viruses, which afflicts thousands of Brazilians every year.

The prevention of diseases transmitted by the *Ae. aegypti* mosquito mainly consists of combating the vector since effective vaccines have not been developed to date. Some of the most efficient insecticides developed for this purpose are organophosphates and pyrethroids, which are very efficient synthetic molecules, but mosquitoes have shown increasing resistance (SILVÉRIO *et al*., 2020). In this way, an interest in more natural approaches to combating the mosquito arose, especially in countries considered biodiversity hotspots, such as Brazil.

Very recently, several Brazilian plants have shown promising results to fight *Ae. aegypti* (MAGALHAES *et al*., 2021; MELO *et al*., 2021; SILVA *et al*., 2021, 2022). In addition, A previous study revealed that *Hiptage benghalensis* (Malpighiaceae) extracts presented promising insecticidal and repellent activity against mosquito vectors (specifically, *Anopheles barbirostris*, *Culex quinquefasciatus*, and *Aedes albopictus*) in South East Asian countries (LALROTLUANGA *et al.*, 2012). Since the Malpighiaceae family has known toxic species, such as *Banisteriopsis caapi*, *Tetrapterys mucronata*, and *Niedenzuella multiglandulosa*, evaluating the toxicity of Malpighiaceae extracts towards *Ae. aegypti* can generate promising results for combating dengue and other neglected diseases that spread in the national territory. In this context, the search for bioactive natural compounds can also benefit from the metabolomic tools previously described, being possible to guide the study towards possible new bioactive compounds.

3 FINAL CONSIDERATIONS: DISCUSSION AND CONCLUSION

The chemistry of natural products has been extensively investigated for decades, and plant secondary metabolites are among the most widely explored. Even though thousands of secondary metabolites have been described for plants, there is still much to be explored, and the sensitive hyphenated techniques and computational tools have proven to be of great value for more comprehensive studies. In fact, studies at a botanical family level are becoming possible, and chemotaxonomic investigations can benefit from these novel approaches.

This study took the Malpighiaceae botanical family as an example of a broad chemical investigation. The investigation began with the development of an analytical method, moved on to chemosystematic studies, and finally, to the search for bioactive compounds. All these investigations included the use of cutting-edge computational tools to guide the studies and answer our initial hypotheses.

The robust chromatographic method developed can be of great use in future studies in Malpighiaceae, and the fast workflow followed to get to these results can inspire future studies to save time and resources in natural products research. One more time, the Quality by Design approach proved to be effective for the analysis of extremely complex samples, such as mixtures of extracts. The use of the molecular networking algorithm and library searches in GNPS allowed us to have a first contact with the tool, and to realize the potential of its use in chemotaxonomy if all the 197 samples were analyzed.

The chemotaxonomic investigations performed in this study made use of cutting-edge computational tools to study Malpighiaceae at a family level. Most of our samples were retrieved from discarded fragmented samples used for DNA extraction in previous molecular studies, and the small amount of sample necessary for our study allowed us to use this material for further chemical analyses. The tools used for MS/MS data processing and analyses expanded the chemical information of Malpighiaceae in the literature, and all the MS/MS datasets generated in this study are publicly available. In fact, the use of open-access software and databases is another plus since our methodology can be reproduced with other taxa (at a family, clade, or genus level).

Correlating the *in silico* classifications with the phylogeny currently accepted for Malpighiaceae enabled a greater evolutionary understanding of the family. The results obtained can guide future studies in Malpighiaceae since we provided evidence of

chemical classes that can be enriched in particular clades. For instance, if future studies want to search for iridoids or benzylisoquinoline alkaloids in Malpighiaceae, they should probably focus on species from clade J (particularly, *Stigmaphyllon* genus). On the other hand, ecdysteroids should be explored in clade H (especially *Niedenzuella* genus), and so on. Further studies should be performed in the following decades to confirm the results obtained with our approach, and the classical methodologies are of great value for such. However, relatively quickly, we were able to carry out this study and propose several chemical classes.

The search for bioactive compounds was also accelerated by using metabolomics tools. The initial bioactivity screening allowed us to select the species with higher larvicidal activity against *Aedes aegypti*, and *Heteropterys umbellata* became the focus of our study. The metabolomic approach effectively directed the isolation of unusual natural nitro compounds, making it possible to infer possible synergistic effects in larvicidal activity and raise hypotheses regarding their ecological roles.

In conclusion, the combination of metabolomic strategies with state-of-the-art analytical techniques, along with cutting-edge computational tools for metabolites annotation, were crucial to leverage Malpighiaceae chemical space and determine a few bioactive compounds. The collaborative research among several research groups enabled this study to be conducted interdisciplinary, making it possible to withdraw more comprehensive conclusions.

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