



Bioprospecting as a strategy for conservation and sustainable use of the Brazilian Flora

Dulce Helena Siqueira Silva^{1*} , Helena Mannocho-Russo¹ , João Henrique Ghilardi Lago² ,
Paula Carolina Pires Bueno³ , Rebeca Previante Medina⁴ , Vanderlan da Silva Bolzani⁵ ,
Wagner Vilegas⁵  & Wilhan Donizete Gonçalves Nunes⁶ 

¹Universidade Estadual Paulista, Instituto de Química, Araraquara, SP, Brasil.

²Universidade Federal do ABC, Centro de Ciências Naturais e Humanas, Santo André, SP, Brasil.

³Universidade Federal de Alfenas, Instituto de Química, Alfenas, MG, Brasil.

⁴Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Cubatão, SP, Brasil.

⁵Universidade Estadual Paulista, Instituto de Biociências, São Vicente, SP, Brasil.

⁶Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Ilha Solteira, SP, Brasil.

*Corresponding author: dulce.silva@unesp.br

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Abstract: In Brazil, research with natural products had a strong impulse when FAPESP supported the creation of the Laboratory of Chemistry of Natural Products of the Institute of Chemistry of USP (1966). In 1999, FAPESP launched the Research Program in the Characterization, Conservation, Restoration and Sustainable Use of Biodiversity (BIOTA-FAPESP), which intensified the sustainable exploitation of biodiversity, and which evolved to form the Biota Network for Bioprospection and Bioassays (BIOprospecTA), which integrates groups from all over the country, optimizing the use of the skills already installed for the bioprospecting of microorganisms, plants, invertebrates, vertebrates and marine organisms. Of the 104 projects related to plant sciences, 35 carried out bioprospection of Brazilian flora, belonging to the areas of Chemistry, Botany, Genetics, Plant Physiology, Plant Morphology, Plant (Chemo)taxonomy, Ecosystem Ecology, Plant Genetics. Physical Sciences, Forest Resources, Forestry Engineering, Agronomy, leading to thousands of publications, engagement of hundreds of students and a deeper understanding of natural products in different biological models through macromolecules analysis aided by computational and spectrometric strategies, in addition to pharmacological evaluations. The development of omics approaches led to a more comprehensive view of the chemical profile of an organism, and enabled integrated and concomitant studies of several samples, and faster annotation of known molecules, through the use of hyphenated and chemometric techniques, and molecular networking. This also helped to overcome the lack of information on the safety and efficacy of herbal preparations, in projects dealing with the standardization of herbal products, according to international standards. The BIOTA-FAPESP program has also focused on environmental aspects, in accordance with the principles of Green Chemistry and has had positive effects on international collaboration, on the number and impact of scientific publications and on partnership with companies, a crucial step to add value and expand the production chain of bioproducts. Also, the compilation, systematization and sharing of data were contemplated with the creation of the NUBBE_{DB} database, of free access, and that integrates with international databases (ACD/labs, American Chemical Society – ACS), helping researchers and companies in the development from different areas of science, technology, strengthening the bioeconomy and subsidizing public policies.

Keywords: natural products, bioactivity, medicinal plants, metabolomics, molecular network, bioeconomy, phytomedicine.

Bioprospecção como estratégia para a conservação e uso sustentável da Flora Brasileira

Resumo: No Brasil, as pesquisas com produtos naturais tiveram um forte impulso quando a FAPESP apoiou a criação do Laboratório de Química de Produtos Naturais do Instituto de Química da USP (1966). Em 1999, a FAPESP lançou o Programa de Pesquisa em Caracterização, Conservação, Restauração e Uso Sustentável da Biodiversidade (BIOTA-FAPESP), que intensificou a exploração sustentável da biodiversidade, e que evoluiu para formar a Rede Biota de Bioprospecção e Bioensaios (BIOprospecTA), que integra grupos de todo o país, otimizando o aproveitamento das competências já instaladas para a bioprospecção de microrganismos, plantas, invertebrados, vertebrados e organismos marinhos. Dos 104 projetos relacionados às ciências vegetais, 35 realizaram a bioprospecção da flora brasileira, em diversas áreas como Química, Botânica, Fisiologia e Morfologia Vegetal, (Químio)taxonomia Vegetal, Ecologia de Ecossistemas, Genética Vegetal, Recursos Florestais, Engenharia Florestal, dentre outros, levando a milhares de publicações, ao engajamento de centenas de estudantes e ao entendimento mais profundo dos produtos naturais em diferentes modelos biológicos por meio da análise de micromoléculas auxiliada por estratégias computacionais e espectrométricas, além de avaliações farmacológicas. O desenvolvimento de abordagens ômicas ampliou a visão sobre perfil químico dos organismos, possibilitou o estudo integrado e concomitante de várias amostras, e a anotação mais rápida de moléculas conhecidas, por meio do uso de técnicas hífenadas, quimiométricas e redes moleculares. Isso também contribuiu para superar a falta de informação sobre a segurança e eficácia dos fitopreparados, em projetos que tratam da padronização de produtos fitoterápicos, de acordo com normas internacionais. O programa BIOTA-FAPESP também tem focado em aspectos ambientais, de acordo com os princípios da Química Verde e teve reflexos positivos na colaboração internacional, no número e no impacto das publicações científicas e na parceria com empresas, etapa crucial para agregar valor e expandir a cadeia produtiva de bioprodutos. Ainda, a compilação, sistematização e compartilhamento de dados foram contemplados com a criação da base de dados NUBBE_{DB}, de livre acesso, e que se integra com bases internacionais (ACD/labs, American Chemical Society – ACS), auxiliando pesquisadores e empresas no desenvolvimento de diferentes áreas da ciência, tecnologia, fortalecendo a bioeconomia e subsidiando políticas públicas.

Palavras-chave: produtos naturais, bioatividade, plantas medicinais, metabolômica, redes moleculares, bioeconomia, fitomedicamentos.

Historical Background

Natural products have played a major role in the healing or improvement of health issues throughout the history of humankind. The first studies of isolation of natural products from plants were described at the beginning of the 19th century. In 1804, the pharmacist Friedrich Wilhelm Adam isolated the morphine from *Papaver somniferum*, a plant used to extract opium and known for soporific and analgesic properties (Barreiro & Bolzani 2009). Morphine was the first secondary metabolite to be commercialized by Merck in 1826, being widely used during World War II and to this day as an analgesic (Cragg et al. 2014).

Pelletier and Caventou studied the dry bark of *Cinchona* species, which Peruvian Indians used to treat some kind of fevers. In 1820, they isolated the quinine, which was the only effective medicine against malaria for almost three hundred years (Pelletier & Caventou 1820). After World War II, quinine, combined with structure-activity relationship (SAR) studies, was responsible for the production of synthetic antimalarials as chloroquine and primaquine (Viegas et al. 2006). In the late 1960s, mainly during the Vietnam War, a rapidly increased mortality was observed due to the loss of effective treatments against malaria, which was mainly associated with the drug-resistant *Plasmodium* parasites, presenting a serious global challenge (Youyou 2015). In 1969, the researcher Tu Youyou became part of a confidential Chinese project to discover novel antimalarial drugs, and she started to collect information on the relevant traditional Chinese medicines. After preparing over a hundred herbal extracts

and conducting several clinical trials, her team found that the ether extract of *Artemisia annua* L., known as Qinghao, showed inhibition of rodent malaria. A few months later, they isolated and characterized a new natural product from this extract, leading to the discovery of artemisinin, which was approved as an antimalarial drug in 1986. In 2015, Tu Youyou won the Nobel Prize in Physiology or Medicine for her discoveries concerning a novel therapy against malaria (Andrade 2015).

The exploration of Brazilian products by Portuguese colonizers was initially focused on *pau-brasil* (*Caesalpinia echinata*), a tree known as “ibirapitanga” to the native Indians, meaning red wood tree. The bright red pigment yielded from its trunk wood is associated with the presence of brazilin, which gives brazilein upon oxidation, which is formed during the extraction procedure. Since that period, this pigment has been used to tint fabrics and as writing ink (Morsingh & Robinson 1970). Besides *pau-brasil*, other pigments from Brazilian lands were explored, such as pigments from *Bixa orellana* (Bixaceae), known as “urucum” (which means red in Tupi language), and *Genipa americana* (Rubiaceae), known as “jenipapo”. *Bixa orellana* seeds are rich in bixin, a norcarotenoid used as a colorant for food and as a sunscreen component, while jenipapo ripe fruits contain genipin, an iridoid used in body paintings by native Indians (Barber et al. 1961, Djerassi et al. 1961).

During the 1600 explorations, Gonzalo Pizarro discovered cinnamon-smelling trees during expeditions in the Amazon basin, and

he thought that the Eastern traders' spices monopoly was finally broken. However, the new species discovered was not *Cinnamomum australe*, the original source of cinnamon found in China and India, but instead, it was *Aniba canelilla* (Lauraceae). The comparative phytochemical studies on such species showed that cinnamic aldehyde is the major component of the true cinnamon bark extract, whereas the major constituent of *A. canelilla* is nitrophenylethane, along with eugenol and methyl-eugenol (Gottlieb & Magalhaes 1959).

The work "Historia Naturalis Brasiliae" represents the first Brazilian natural history compendium. It is the result of observations made during important scientific expeditions in the 18th century for the European researchers Georg Marcgrave, Johannes de Laet, and the physician Willem Piso, who contributed to the knowledge on medicinal plants from South America. The arrival of the Royal Court to Brazil in 1808 promoted Brazilian science, since several scientific expeditions arrived in the country to study our fauna and flora. The pharmacist Ezequiel Correia dos Santos obtained pereirin in 1838 from *Geissospermum leavis* (Apocynaceae) barks, a medicinal plant known as "pau-pereira", "quinarana" or "pau-forquilha", and used to treat fever and malaria (Pinto et al. 2002). In 1847, the German pharmacist Theodore Peckolt was invited to join the effort to study Brazilian flora, being a pioneer in the systematic study of medicinal plants. One of his remarkable works was the isolation of plumeride (formerly named agoniadin) from *Plumeria lancifolia* (Apocynaceae), a medicinal plant used by the Guarani Indians to treat malaria. The structural elucidation of this substance only occurred 88 years later, and it is considered the first isolated iridoid from a natural source (Santos et al. 1998).

In the early 1900s, the systematic study of Brazilian plants received a strong boost with the foundation of the Institute of Agricultural Chemistry, in Rio de Janeiro, which has been recognized as the birthplace of the studies of Natural Products in Brazil. This institute counted with remarkable researchers such as Otto Richard Gottlieb, Walter Baptist Mors, Benjamin Gilbert, Mauro Taveira Magalhães, and Roderick Arthur Barnes, all of them with national and international prestige. After its extinction in 1962, different research groups dedicated to the field of Natural Products were consolidated, especially in Rio de Janeiro and São Paulo. In Rio de Janeiro, the Natural Products Research Center (NPPN, now CPPN), linked to the Pharmacy School of the Federal University of Rio de Janeiro (UFRJ), was created by Walter Mors. In São Paulo, Otto Gottlieb was invited by FAPESP to organize a Natural Products laboratory at the Institute of Chemistry of the University of São Paulo (IQ-USP) (Pinto et al. 2002), after a short period (1962–1965) at the University of Brasília (UnB), where he established an organic chemistry laboratory, but left as an act of protest after the military coup of 1964 and government intervention, which led to the dismissal of several professors (Faria 1997).

The Laboratory of Natural Products Chemistry at the IQ-USP, led by Otto Gottlieb and Paschoal Senise, was created in 1966, supported by FAPESP through a project of the Initiatives modality, which provided funding to establish a complete natural products laboratory. These researchers collaborated with several public institutions across the country, including Museu Paraense Emílio Goeldi and the Federal Universities of Ceará, Paraíba, Alagoas, and Minas Gerais, and the Rural University of Rio de Janeiro. The intensive formation of human resources resulted in the nucleation of several natural products research groups all over Brazil and also in Latin American countries and Portugal.

The main target addressed by the IQ-USP research team at that time was the Amazon biome and plant species, mainly from Lauraceae and Myristicaceae families. Such studies yielded more than 1,000 new compounds, including neolignans, a novel class of natural products structurally related to the lignans. In addition, they contributed to understanding the relationship among morphology, the evolution of plants and their metabolism, as well as their taxonomy based on the plants chemical profile – also called chemosystematics (Fioravanti 2020, Biblioteca Virtual da FAPESP 2022).

In 1999, FAPESP launched the Research Program in Characterization, Conservation, Restoration and Sustainable Use of Biodiversity (BIOTA-FAPESP) with the main objective of mapping and analyzing the biodiversity of microorganisms, animals and plants of São Paulo State. In addition, the program also aims at the development of value-added natural products which might trigger bioeconomy at regional and macro-scale levels with potential positive impact on local populations, who retain traditional knowledge, through benefit-sharing from the sustainable exploration of biodiversity, in the scope of bioeconomy premises. In this way, it would bring returns to the society by means of novel therapeutic agents, agroceuticals and further useful products, as well as the formulation of policies aimed at biodiversity conservation. BIOTA-FAPESP currently involves more than 1,200 professionals, including 900 researchers and students from São Paulo, 150 collaborators from other Brazilian states, and more than 80 international collaborators (FAPESP 2022). The scientific content produced by the BIOTA-FAPESP Program (www.biota.org.br) may be assessed in open databases by national and international scientific communities. In addition, the standardized collections of plants and animals carried out in the São Paulo State with geographic coordinates information can be consulted in the Environmental Information System, SinBiota (<http://sinbiota.biota.org.br>), based on the scientific name, the collector name, the location, or the date of the collection, which greatly contributes to research data reproducibility. The Biota Program evolved further to develop the Biota Network of Bioprospection and Bioassays (BIOprospecTA – www.bioprospecta.org.br), which integrates research groups mainly from São Paulo State working with the prospection of bioactive compounds from microorganisms, plants, invertebrates, vertebrates, and marine organisms (FAPESP 2022).

Along with the BIOTA program, FAPESP boosted the Natural Products Chemistry in Brazil through the creation of the Thematic projects modality in 1990, the Research, Innovation and Diffusion Centers (CEPID) in 2000, in addition to the Young Researcher (JP) modality in 1996, which strongly contributes to the establishment of state-of-the-art laboratories and research lines in the scope of biodiversity in São Paulo State. From the JP projects linked to BIOTA, 25 have been concluded, and four are currently being developed. Thematic, CEPID and JP projects support long-term and multidisciplinary research, involving several researchers and students from different departments or institutions, with the purpose of obtaining scientific, technological, and socioeconomic results. So far, 95 Thematic projects related to the BIOTA-FAPESP program have been approved, 27 are in progress and 72 have been concluded (Biblioteca Virtual da FAPESP 2022). The Center of Research and Innovation in Biodiversity and Drugs (CIBFar) is one of the CEPIDs funded by FAPESP and aims to develop basic and applied science based on research related to natural products, synthetic organic chemistry, molecular and structural biology, biochemistry, in addition

to medicinal chemistry, drug design, and pharmacological assays. This center involves laboratories in five different institutions, including the State universities in São Paulo (CIBFar 2022).

BIOTA-FAPESP Survey of Projects Involving Plant Species

Over 20 years, the BIOTA-FAPESP Program has provided research grants to 324 proposals distributed among different fields of knowledge (data retrieved from the Fapesp Virtual Library on January 4th, 2022) (Supplementary File, Sheet 1). These fields include Biological Sciences (64.8%), Physical Sciences and Mathematics (17.0%), Agronomical Sciences (9.9%), Humanities (2.8%), Interdisciplinary Subjects (2.2%), Health Sciences (2.2%), and Applied Social Sciences (1.2%). Additionally, 13 fellowships were awarded in the fields of Biological Sciences, Physical Sciences and Mathematics, Agronomical Sciences, and Health Sciences. From those proposals, 104 projects (32.1%) and two fellowships (15.3%) comprising research involving specifically plants were provided (Figure 1) (Supplementary File, Sheet 2).

A wordcloud of the keywords provided for each of these 104 projects is depicted in Figure 1A, which evidences a high frequency of “Biodiversity”, “Cerrado”, “Atlantic Forest”, “Natural Products” and “Medicinal Plants” used as keywords. Most of the projects related to plant species (Supplementary File, Sheet 2) belong to Biological Sciences (62.3%), with Botany and Ecology the main field of knowledge of the proposals (74.2%), followed by Genetics (12.1%). Those three main areas included studies primarily on the subjects of Plant Physiology, Plant Morphology, Plant Taxonomy, Ecosystems Ecology, and Plant Genetics. Physical Sciences and Mathematics comprised 20.8% of the projects, in which Chemistry represented 77.3% of those proposals, with Organic Chemistry being the main studied subject. Agronomical Sciences corresponded to 13.2% of the proposals, being Forestry Resources and Forestry Engineering and Agronomy the main studied fields of knowledge from that main area. Finally, it is worth highlighting Health Sciences, with 3.8% of the proposals. All projects retrieved under this main area were related to Pharmacy and Pharmacognosy, with emphasis on the study of natural products and medicinal plants, being interdisciplinary projects closely related to the Chemistry field. It is important to emphasize that a significant part of the proposals have not specified the subject of the study; in these cases, they were listed as “Not Specified” in Figure 1B. This highlights the importance of specifying every field regarding information of the proposals, in order to make it possible to perform such comparisons more accurately.

The outer ring in Figure 1B indicates the institution to which each proposal was attributed by the principal investigator of the project, and it is possible to observe a correlation between the prevalence and participation of some research institutes according to the main field of study. For example, studies focused on Botany, Genetics and Ecology were mainly carried out in Institutes of Biology distributed in the São Paulo State, including those belonging to public universities, whereas studies focused on Chemical Sciences were carried out mainly at Institutes of Chemistry and Pharmacy Schools.

From the 104 projects related to plant sciences (Supplementary File, Sheet 2), 23 projects addressed natural products and/or bioprospection of the Brazilian flora, all of them in the context of the Chemistry and

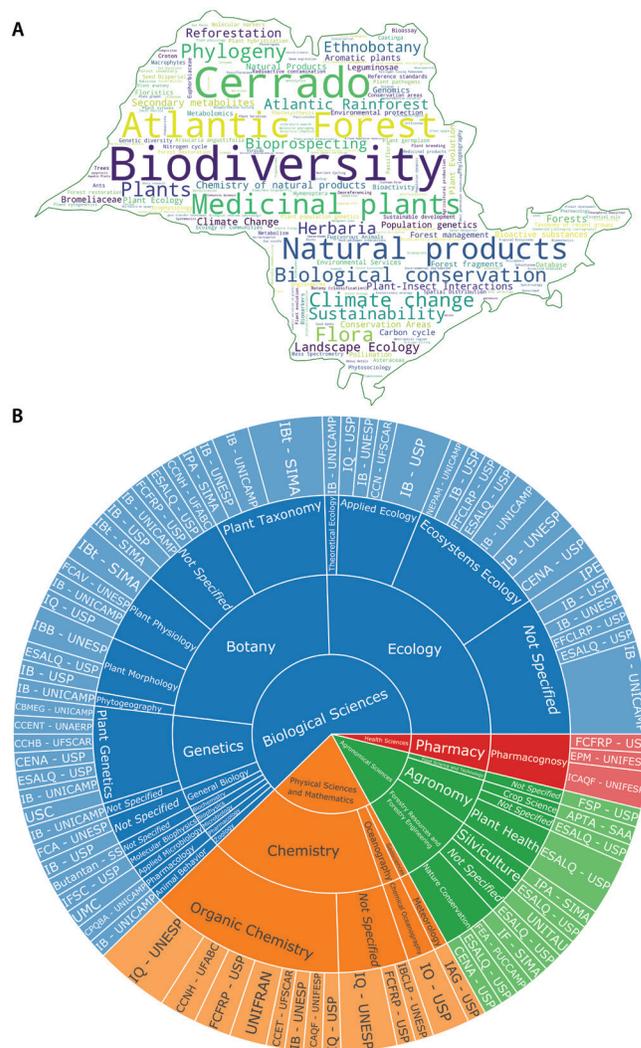


Figure 1. (A) Wordcloud based on the top 200 most frequently used keywords used in the 104 proposals involving studies on plant species. (B) Multilevel pie chart showing the distribution of proposals involving plant species financed by the BIOTA-FAPESP Program (Supplementary File, Sheet 2). The 104 research projects related to plant species were filtered from the complete project list exported from the Fapesp Virtual Library (<https://bv.fapesp.br/>, retrieved on January 4th, 2022) by checking keywords related to plant species in each project subject. A further check was performed by searching the following descriptors on the summary or project title: phytochemistry, natural product, medicinal plant, vegetal species, plant species, metabolomics, bioprospection, bioactive compound, bioactive substance, active substance and herbal. Plant interactions with microorganisms (viruses, fungi, and bacteria) were also included; however, proposals focused on microorganisms only were excluded as they have been addressed in another article of this Biota Neotropica edition.

Pharmacy areas. To the best of our knowledge, 450 articles officially assigned – by means of citation of the Fapesp proposal number – under at least one of those 23 projects were published from 2001 to 2021 (Supplementary File, Sheet 3). Articles that have been produced within these projects, but were not officially linked to the BIOTA-FAPESP program, were not considered due to the lack of precise information. This emphasizes the relevance of adding information on the funding agencies and proposal number in every submitted article. With impact factors (JCR, 2020) ranging from 0.961 to 54.908, and citations reaching up to 1480 (data retrieved from Crossref on January 16th, 2022), those publications supply a consistent overview of the results and evolution

of research performed over 20+ years within the BIOTA-FAPESP program (Figure 2A). It is possible to observe the great number of articles published in recent years compared to the beginning of the BIOTA Program, highlighting the growth in funded proposals in the field of plant natural products.

The proposal entitled “Conservation and sustainable use of the plant biodiversity from the Cerrado and the Atlantic Forest: chemical diversity and prospecting for potential drugs” (FAPESP #98/05074-0), carried out at the Institute of Chemistry of Araraquara (IQ-UNESP) and partner research groups, focusing on the chemistry of plant species, was the first financed by the BIOTA-FAPESP Project to be successfully carried out. Aiming to search for bioactive compounds from plant species occurring in remnant Cerrado and Atlantic Forest areas of the São Paulo State, it included the screening of plant extracts through phytochemical techniques and bioassays in order to prospect cytotoxic, antioxidant, antifungal and antimalarial compounds. A plethora of plant families, plant species, and chemical compounds classes were studied with the aid of 38 associated scholarships and two associated grants from 1998 to 2004. From this project, 16 research articles were formally reported from 2001 to 2014 at the Fapesp Virtual Library, and one patent was deposited in 2003, although more than 90 scientific publications and 10 patents have been retrieved addressing subjects included in the project goals and carried out by its researchers. Such data evidence the need for actions towards the systematic association of published results to the research funded by Fapesp, which has greatly improved in more recent years.

Currently, 72 proposals are being carried out in the BIOTA-FAPESP Program, with 21 of them concerning the study of plant species or plant interactions, and five of them comprising Chemistry or Pharmacy. “Inventorying secondary metabolism applying metabolomic strategies: contribution to the Brazilian biodiversity valuation” (FAPESP #20/02207-5) is the most recently approved proposal within BIOTA-Fapesp and is currently being developed at the School of Pharmaceutical Sciences of Ribeirão Preto (FCFRP-USP). The proposal aims to establish a comprehensive understanding of natural products in different biological models through the universal analysis of small molecules aided by computational and spectrometric strategies, in addition to pharmacological evaluations.

A series of National Institutes of Science and Technology (INCTs), supported jointly by the National Council for Scientific and Technological Development (CNPq) and State Research Funding Agencies (FAPs) since 2008 has triggered a marked development in most scientific areas. It is worth mentioning the INCT-BioNat on Biodiversity and Natural Products (FAPESP #14/50926-0), which was approved in 2014 and is dedicated to investigating a broad range of subjects within the area of natural products. This program has been carried out at the Institute of Chemistry – UNESP and several natural products research groups throughout Brazil, focusing on the creation of a national research network to map the chemical and biological information regarding terrestrial, marine and microorganisms species from the Brazilian biodiversity. So far, this project has resulted in 89 publications reported at Fapesp Virtual Library, being the most productive proposal so far funded by BIOTA-FAPESP among the ones focused on chemical and pharmacological studies of natural products from the Brazilian flora.

It is also relevant to highlight the impact of international collaboration on the number of scientific publications (Figure 2B) through the years. Despite the growth in the number of articles (regardless of international co-authors) in the past years, the publication rate of studies solely represented by Brazilian groups increased more than those with international collaboration. This difference may be due to the INCT Bionat project, which includes and encourages collaborations among research groups from São Paulo State and from other Brazilian regions. Also, it is worth noting a significant decrease in publications with international collaborations in 2021, possibly due to the COVID-19 pandemic. In 2020 and 2021, several projects had to be readjusted to the world’s new reality, which included international travel restrictions.

Regarding the publications in the plant natural products field involving researchers from around the globe, it is possible to observe that these collaborations are concentrated in groups from the Americas and Europe – especially the United States, the United Kingdom, and Germany (Figure 2C). Few articles include researchers from Africa, Asia, and Oceania, although those continents comprise biodiversity hotspots. Finally, the evolution of impact and citations of such articles through the years evidence a marked increase in the number of high-impact publications, mainly after 2016 (Figure 2D), involving international collaborations. This analysis corroborates the importance of establishing collaborations worldwide with internationally renowned laboratories acting in the frontiers of knowledge. In this way, national groups can benefit from the acquired knowledge, and the novel techniques and methodologies that students and postdoctoral scholars bring back and establish in Brazilian laboratories after their period abroad.

Chemical and Biological Studies Regarding Brazilian Flora in BIOTA-FAPESP

São Paulo State comprises two main biomes which differ strongly regarding soil, climate, humidity, forest composition, among others. Although many species may occur both in Cerrado and Atlantic Forest, the chemical profile of a particular species may vary depending on the collection site among other factors, such as seasonality and even day period due to the plant’s circadian rhythm. Such observations have triggered interesting ecophysiological studies which evidenced infraspecific chemical variability as observed in *Casearia sylvestris* (Bueno et al. 2021) and in the essential oil of leaves of *Cryptocarya mandiocana* (Telascrea et al. 2007). Further interesting examples regarding ecophysiological interactions include those associated with chemical biology of plant-insect-parasites trophic systems, as in the study of the neotropical shrub *Piper amalago*, which showed how herbivory, caterpillar biodiversity and plant-herbivore network structure might be affected by the chemical profile and structural dimensions of phytochemical diversity (Cosmo et al. 2021).

Investigations on other biomes, such as the Amazon Forest and Rupestrian Fields, gave additional examples of the huge chemodiversity of Brazilian plant species, representing a potential source for value-added products. Some examples include the Amazon tree *Calycophyllum spruceanum*, with reported folk uses by Brazilian indigenous populations and rich in seco-iridoids and antioxidant phenolic derivatives (Zuleta et al. 2003), and *Syngonanthus nitens* and other ‘sempre-vivas’ species, which disclosed chemical profiles rich in xanthones, naphthoquinones and flavonoids (Pacífico et al. 2011).

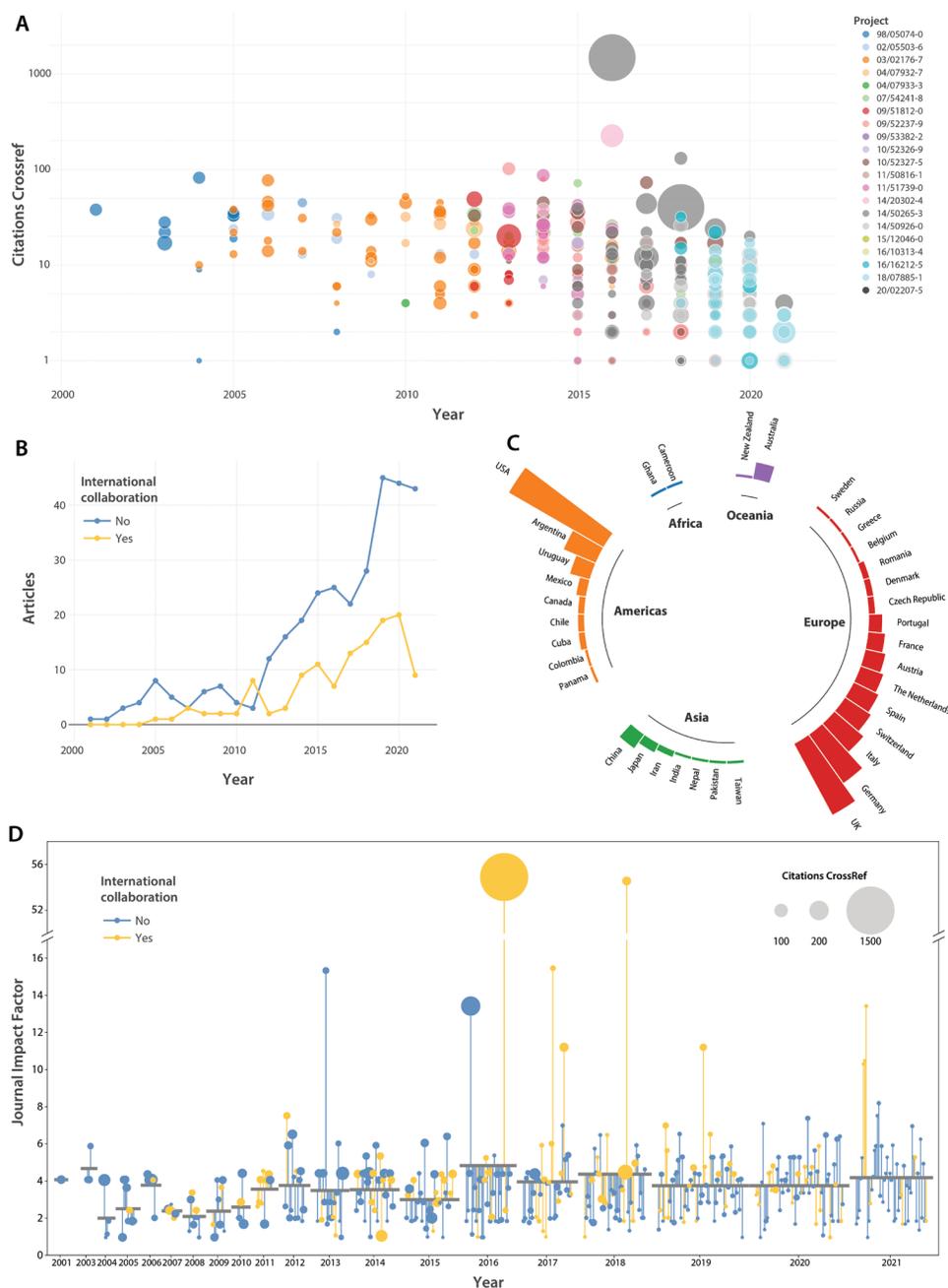


Figure 2. Analyses resulting from the survey of articles published over 20 years within the BIOTA-FAPESP Program focusing on natural products and bioprospection of Brazilian flora. (A) Overview of the publications resulting from the 23 approved proposals. The size of each circle corresponds to the journal impact factor according to the Journal Citation Reports (JCR, 2020). Two recent proposals without publications to date have not been displayed. The y-axis is a logarithmic scale of the citations retrieved from Crossref, therefore, articles without citations until January 16th (2022) have not been displayed. (B) Number of articles published with or without international collaborations over the years. (C) Countries involved in collaborative research within Biota-Program projects. (D) Impact of international collaboration on publications over the years. The thicker horizontal lines correspond to the average of all the publications in each year. The size of the circles is relative to the citations of each article.

Nevertheless, the main focus of most of the projects within the BIOTA-FAPESP Program addressed Cerrado and Atlantic Forest plant species. Several articles regarding the chemistry of natural products published in this scope involve classical methodologies, which mainly comprise the isolation, characterization, and biological evaluation of purified and/or semi-synthetic compounds. This kind of approach led to several important discoveries of bioactive and/or novel natural products, including amides, alkaloids, benzoic acids, chromenes, coumarins,

terpenoids, flavonoids, lignoids, polyketides, peptides, fatty acids and derivatives, in addition to mixed-origin secondary metabolites, which reinforces the fantastic chemodiversity of Brazilian plants. Based on the data described in the 450 research articles which resulted from projects addressing plant species secondary metabolism between 2001 and 2021, 230 papers comprise biological/pharmacological approaches conducted with natural compounds. These biological evaluations consists of antiparasitic (33.0%), antitumoral (23.9%), antibacterial

(11.7%), antioxidant (8.7%), antifungal (6.1%), anti-inflammatory (5.2%), antiulcer (2.2%), acetylcholinesterase inhibitor (1.7%), anxiolytic (1.3%), anti-photoaging (0.9%), antinociceptive (0.9%), cathepsin inhibitor (0.9%), anti-AIDS (0.4%), anti-Alzheimer (0.4%), anti-COVID (0.4%), antidiabetic (0.4%), antitubercular (0.4%), COX inhibition (0.4%), NO production inhibition (0.4%), and photosynthesis inhibition (0.4%) (Figure 3).

More than 50% of all studies involving the prospection of bioactive natural products focus on the discovery of antitumoral and antiparasitic compounds (Figure 3). This could be explained, at least in part, by the increasing necessity of discovering more potent and less toxic antitumoral chemotherapeutic agents – including those based on unprecedented structures of natural products. For instance, different cyclotides were isolated from the roots of *Pombalia calceolaria*, and their effects on breast cancer cell lines were evaluated (Pinto et al. 2018). Another example involves the isolation of chemically related neolignans from *Nectandra leucantha* active against human melanoma resistant cells, caused by apoptosis and necrotic mechanisms (de Sousa et al. 2019).

Infections caused by protozoan parasites, known as Neglected Tropical Diseases (NTD), such as Chagas disease, leishmaniasis, schistosomiasis, and malaria, are a major cause of disease and mortality in many tropical countries, especially in Brazil. The current therapies for such diseases are highly toxic and promote severe side effects, and the discovery of novel active principles and/or lead structures for the development of new drugs against such diseases is hence an extremely urgent task. This could be the main reason why several Brazilian research groups are focused on the discovery of new lead compounds for NTD treatment. For example, piperlongumine from *Piper tuberculatum* and biomimetic derivatives displayed activity against *Leishmania infantum* and *L. amazonensis* (Moreira et al. 2018). A study performed with *Piper gaudichaudianum* afforded gaudichaudianic acid as a potent trypanocidal compound. After resolution, the (+)-enantiomer

showed higher activity than its antipode, whereas mixtures of both enantiomers disclosed a synergistic effect, with the racemic mixture being the most active (Batista et al. 2011). Additionally, the assessment of the antimalarial activity of anacardic acid and cardol derivatives isolated from *Anacardium occidentale* evidenced a promising potential against *Plasmodium falciparum* (Gimenez et al. 2019). Also, the anti-schistosomal activity of ent-kaurane diterpenoids isolated from *Baccharis lateralis*, were investigated and displayed promising *via oral* effects in *in vivo* assays (Sessa et al. 2020).

Classical bioprospecting approaches, such as the discovery of antibacterial, antioxidant, antifungal, and anti-inflammatory compounds, were widely investigated in the projects developed during the first decades (2000–2020) of the BIOTA-FAPESP program. Such studies included the investigation of medicinal plants for the confirmation of alleged bioactivities by establishing their chemical profiles and detection of secondary metabolites, which might corroborate their folk use. On the other hand, these investigations might also alert against dangerous side effects, or even detect possible adulterations. For instance, neuro disorders reported by patients suffering from chronic kidney disease after ingesting star fruit were successfully attributed to its caramboxin content (Garcia-Cairasco et al. 2013), and the chemical profile of *Maytenus* species, used as a folk medicine to treat gastric ulcer, was established in order to differentiate them from other species with similar phenotypic features (Coelho et al. 2003).

Several edible fruits have also been investigated in the context of GRAS (“generally regarded as safe”) plants, in the search for bioactive constituents which might lead to value-added products e.g. nutraceuticals. The detection of biflavonoids in *Garcinia brasiliensis* (Arwa et al. 2015), gallic acid derivatives in *Spondias tuberosa* and *Inga laurina* (Zeraik et al. 2016, Falcoski et al. 2021), and anthocyanins in *Eugenia jambolana* (Dametto et al. 2017) with chemopreventive activity indicated a promising potential for their sustainable exploration. In addition, the search for value-added products in fruits residues (inedible

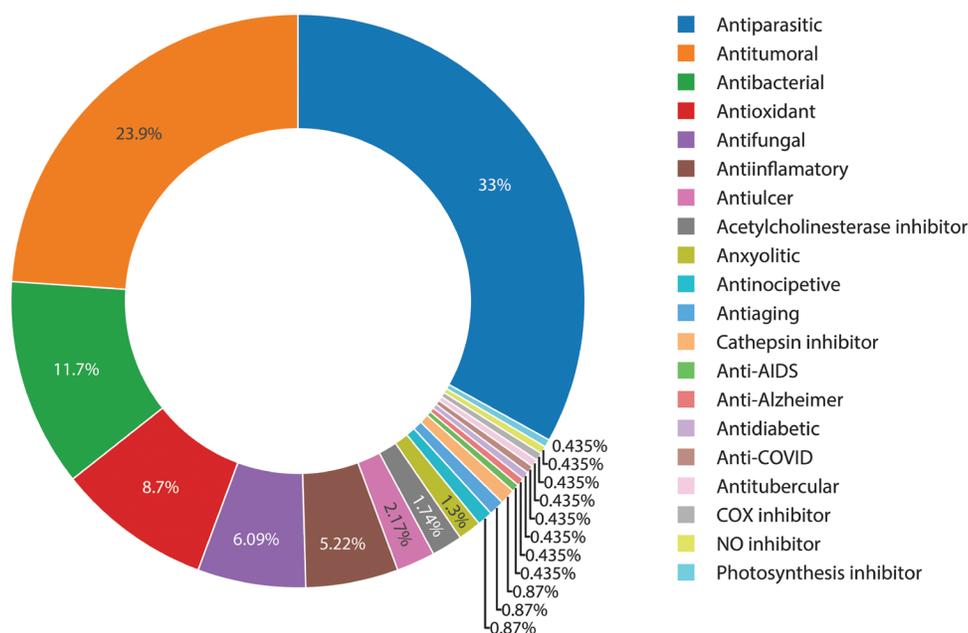


Figure 3. Bioprospecting studies addressing biological and/or pharmacological properties of natural products isolated from plants collected in Brazilian biomes in the scope of BIOTA-FAPESP projects (2001–2021).

parts), led to the detection of biflavonoids in *Platonia insignis* bark and seeds extracts, including the selective extraction of morelloflavone, which has been reported for several important bioactivities, including chemoprevention (Ribeiro et al. 2021).

Such studies were also important to evidence the experience of Brazilian research groups for partnerships with companies, such as Eurofarma, Grupo Centroflora/Anidro do Brasil S.A., Apsen Farmacêutica and Natura & Co Group, aiming at the development of specific studies which might lead to value-added products from the Brazilian biodiversity. Such efforts were partially supported by Federal agencies such as FINEP and CNPq, e.g. through research fellowships RHAÉ, which resulted in the establishment of the chemical profile and chemical markers of several medicinal plants as *Phyllanthus niruri* (“quebra-pedra”), *Myrcia uniflora* (“pedra ume-caá”) and *Ptychopetalum olacoides* (“marapuama”) (Anvisa 2017). Additional studies included the investigation of *Stemodia spectabilis*, used as a folk medicine to treat respiratory tract infections (Silva et al. 2002), and *Cassia spectabilis*, used to treat gastric disorders, which disclosed piperidine alkaloids with anticholinesterase and antiinflammatory properties (Viegas et al. 2007). Despite such efforts, only a few herbal medicines from Brazilian plants are commercially available to date, which had their development thoroughly financed by pharmaceutical companies. A chemically standardized extract of *Cordia verbenaceae* rich in sesquiterpenes was used as the basis for the development of Acheflan[®], used as a topical anti-inflammatory. Additional examples include Sintocalmy[®], an anxiolytic standardized herbal medicine prepared with extracts of *Passiflora incarnata* containing the bioactive flavonoid vitexin, and Fitoscar[®], an ointment containing *Stryphnodendron adstringens* extracts, traditionally used in wound healing, and rich in phenolic derivatives, mainly condensed tannins.

More recently, the development of powerful techniques and equipment triggered a paradigm shift in the focus of current research on natural products. The classical approaches have been replaced by broader scope studies involving state-of-the-art *omic* strategies, enabling integrated studies of several samples at a time and faster annotation of known compounds through the use of hyphenated techniques and chemometrics.

Even though research involving classical methodologies has proven highly important for the chemistry of natural products and bioprospection fields, this time-consuming and costly approach can often lead to the re-isolation of well-known natural products. In this way, the development of highly sensitive detection techniques and computational tools in recent years caused a revolution in the field. Particularly for sensitive hyphenated techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS), which allow the chromatographic separation of compounds prior to the detection, and can use small amounts of sample, enabling the detection, analysis, and annotation of compounds in very low concentrations. Similarly, NMR spectrometers with a higher magnetic field (≥ 950 MHz), running with state-of-the-art electronics and equipped with cryoprobes (which decrease instrumental noise by increasing sensitivity by up to 16 times), allow the analysis of samples at a microgram level due to the increased sensitivity of experiments (Pilon et al. 2020, Valli et al. 2019).

Such modern approaches have also been used in the study of *Lippia sidoides* (“alecrim pimenta”), a medicinal plant included in

the RENISUS for its anti-inflammatory, antifungal and antiparasitic properties (Rensisus 2022). Due to various taxonomic problems involving some *Lippia* genus (Verbenaceae), the metabolite profiling of fifteen extracts of various organs from six *Lippia* species was performed and compared using UHPLC-PDA-TOF-MS and chemometric tools, which enabled efficient peaks annotation. Moreover, dereplication results and hierarchical data analysis disclosed chemical similarities for four *Lippia* species which contributed to highlight its chemotaxonomical relevance (Funari et al. 2012).

In the study of Camaforte and colleagues (2019), the authors investigated the hypoglycaemic and hypolipidaemic effect and the molecular mechanism of action of *Bauhinia holophylla* (Fabaceae), a medicinal plant that has traditionally been used to treat diabetes. Besides the investigation of biochemical parameters such as glucose tolerance, insulin sensitivity, blood parameters, gene and protein expression, and the *in vivo* and *in vitro* inhibition of intestinal glucosidases, authors used hyphenated techniques such as FIA-ESI-IT-MS/MSⁿ and HPLC-PAD-ESI-IT-MS to certify an authentic *B. holophylla* extract by analyzing flavonoid derivatives of quercetin, myricetin, luteolin, and kaempferol (Camaforte et al. 2019). Similarly, Saldanha and colleagues (2013) used a combination of HPLC-PAD-ESI-ITMS, FIA-ESI-IT-MSⁿ, and NMR to analyze 24 flavonoids derivatives and phenolic acids in the hydroalcoholic extract of *Myrcia bella*, six of them tentatively identified through retention time values, co-chromatography with authentic samples, UV and MS spectral data, and eighteen of them isolated and characterized by UV, MS, and NMR spectral data (Saldanha et al. 2013).

These high-resolution separation and analytical techniques, including combinations such as LC-MS/MS, UHPLC-TOF-MS, CE-MS/MS, LC-NMR, or LC-DAD-SPE-NMR-MS, along with new algorithms, cheminformatics, and bioinformatics tools, enabled significant advances in the study of plant natural products. It provided the development of powerful and versatile approaches, which boosted the annotation, identification, and quantitation of metabolites (Bueno & Lopes 2020). By comparing mass spectrometric data from compounds present in a given extract with previous spectrometric reports, it is possible to dereplicate and annotate known compounds or the compound class of a particular metabolite. In this way, the isolation of compounds can be more rationalized towards possible novel natural products, widening the inspirational sources for drug design, for instance, from a higher chemodiversity pool.

Several spectrometric databases were developed to allow these investigations in a semi-automated fashion. Among those, the Golm Metabolome Database (GMD), the Automated Mass Spectral Deconvolution Software (AMDIS), and the National Institute of Standards and Technology libraries (NIST) can be highlighted (Pilon et al. 2020). The GC-MS NIST libraries, for example, have been widely used for the annotation of natural compounds for several decades, being particularly useful for the analyses of essential oils extracted from plants. Several articles published in BIOTA-FAPESP have used this approach to investigate the chemicals present in dozens of essential oils with a variety of bioactivities (Aguiar et al. 2013, Bou et al. 2013, Oliveira et al. 2015, Soares et al. 2017).

More recently, a worldwide community effort to develop the Global Natural Products Social Molecular Networking (GNPS) platform (Wang et al. 2016) was performed, including researchers from more than ten countries, including Brazil. This free platform gathers mass

spectrometry data (from both GC–MS and LC–MS instruments) from a variety of samples, including plant metabolites, and it has been widely used as a dereplication strategy in the chemistry of natural products field (Yang et al. 2013). In addition to the public spectral libraries, GNPS enables researchers to visualize the mass spectrometry data obtained (e.g. from a natural source) as molecular networks composed of nodes and edges. Each node in a molecular network corresponds to an MS/MS spectrum, while the edges connecting two nodes correspond to the spectral similarity between them. In this way, closely correlated MS/MS spectra will most likely be connected, indicating similar structures. These networks enable the visual inspection of the whole chemical information retrieved in such analysis, being particularly useful for annotating possible analogs (Wang et al. 2016). This platform has been widely used worldwide in several research fields, including in the BIOTA-FAPESP projects.

Two recent examples illustrate such an approach and highlight important advances in plant metabolome dereplication studies. The first one reports the use of molecular networking and library searches to annotate a total of 61 compounds from nine Malpigiaceae species collected in different Brazilian locations (Mannocho-Russo et al. 2020). Leaves from *Byrsonima intermedia*, *Mcvaughia bahiana*, *Barnebya harleyi*, *Ptilochaeta densiflora*, *Bunchosia maritima*, *Hiraea restingae*, *Niedenzuella multiglandulosa*, *Banisteriopsis laevifolia*, and *Amorimia septentrionalis* were used in the study which proved an efficient approach for investigating complex matrices. In this way, O-glycosylated flavonoids, C-glycosylated flavonoids, quinic and shikimic acid derivatives, sterols, and other phenols, were successfully

annotated after chromatographic method development by Quality by Design and statistical analysis (Figure 4). Similarly, molecular networking as a dereplication strategy for metabolite annotation was also used to investigate the chemical diversity of *Selaginella convoluta*, a desiccation tolerant plant native to the Brazilian semiarid region (Caatinga). The molecular networking approach allowed the putative annotation of 39 metabolites, including known selaginellins (known as *S. convoluta* chemical markers) and flavonoids, besides three unprecedented selaginellin derivatives (Reginaldo et al. 2021).

In addition to the dereplication and annotation of metabolites, these sensitive analytical techniques have proven valuable for metabolomic studies, allowing a more comprehensive overview of the metabolites (usually in the range of 50 to 1500 Da) produced by a specific organism. As a consequence, one can investigate if and how groups chemically differentiate as a response to biotic stimuli, stress, environmental changes, among others. These studies can be broadly classified as targeted or untargeted, in which the first one will focus on possible chemical changes in pre-selected compounds, whereas the second one will look at the whole data aiming to annotate and/or quantify as many compounds as possible (Brunetti et al. 2018, Pilon et al. 2020, Bueno & Lopes 2020).

Studies on ecological interactions of non-model plants in their natural habitat have been boosted by metabolomics under the BIOTA-FAPESP program. As reviewed by Brunetti and colleagues (2018), the technological and analytical advances in the fields of chemistry and biology, i.e., genomics, transcriptomics, proteomics, and metabolomics, have enabled the analysis of the molecules involved in such interactions (Brunetti et al. 2018).

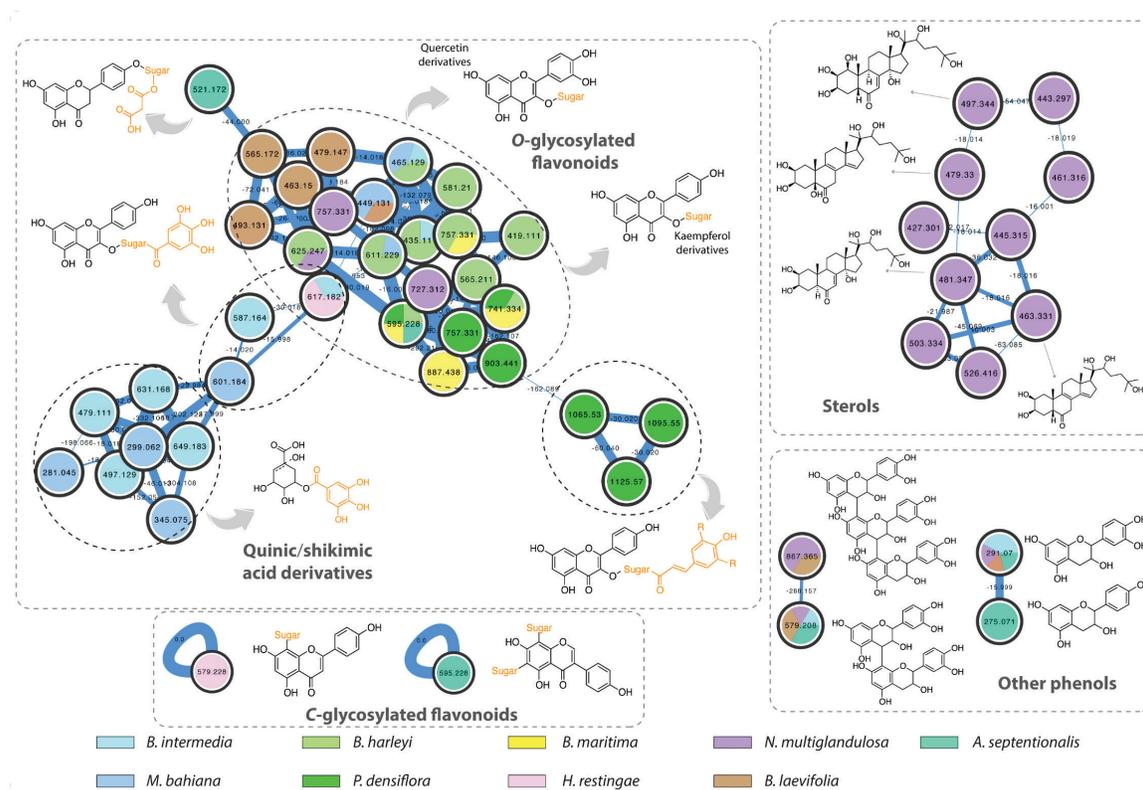


Figure 4. Molecular networks and classes of compounds annotated in the study conducted with Malpigiaceae species. Structures shown with a defined configuration were confirmed with standards isolated from *N. multiglandulosa*. Reprinted with permission from (Mannocho-Russo et al. 2020). Copyright 2020 American Chemical Society.

For instance, the plant-insect interaction between *Tithonia diversifolia* (Asteraceae) and *Chlosyne lacinia* (Nymphalidae) was investigated by untargeted LC-MS/MS-based metabolomics and molecular networking for metabolite annotation in the study of Gallon and colleagues (2019). Following the untargeted metabolomics approach, 28 substances, including sesquiterpene lactones, flavonoids, and lipid derivatives were putatively annotated in *T. diversifolia* leaves and in *C. lacinia* feces, larvae, pupae, butterflies, and eggs. Their findings also contributed to disclosing the ecological interaction among the annotated metabolites. Although typical sesquiterpene lactones are known as defensive metabolites in *T. diversifolia*, intact sesquiterpene lactones were excreted by *C. lacinia* larvae, suggesting a possible strategy to reduce the bioavailability of such compounds in the digestive system (Gallon et al. 2019).

In another study from Saldanha and colleagues (2020), authors investigated the specialized metabolism and the genetic diversity of the medicinal plant species *Myrcia bella* (Myrtaceae), a Brazilian native plant from Cerrado areas, using LC-HRMS-based metabolomics approach and multivariate data analysis. Following this approach, 271 samples of *M. bella* from seven regions were chemically profiled and the chemical markers selected by molecular network were annotated using an *in-silico* database of natural products. Seasonal variations, genetic diversity, chemotypes and environmental factors were then correlated with the metabolomics data, which pointed out the impact of abiotic stresses and genotype on the accumulation of some secondary metabolites (Saldanha et al. 2020).

It is important to point out that those multilevel data require additional processing power to help interpret the interactions between organisms or to unveil, for example, biological properties of active compounds and biological targets. Indeed, metabolomic studies count on several data processing steps besides statistical analyses, which can be univariate or multivariate. The multivariate analyses can be supervised, such as partial least squares discriminant analysis (PLS-DA), or non-supervised, such as Principal Component Analysis (PCA). On the other hand, the univariate analyses 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).

In the study of Sousa and colleagues (2020), for example, multivariate statistics and machine learning were used to analyze the structure-activity relationships and the biological activity against murine (B16F10) and human (A2058) melanoma cells potential of 23 semi-synthetic neolignan derivatives from the Brazilian plant species *Nectandra leucantha* (de Sousa et al. 2020). Another interesting example involving the use of multivariate data analysis was applied in the study of the chemical composition and biological activities of two different populations of *Guarea macrophylla* (Meliaceae) (Oliveira et al. 2019). The authors investigated the leishmanicidal and cytotoxic potential of the essential oils from leaves and correlated the bioactivities with the chemical profile. The GC-MS of essential oils from leaves of *G. macrophylla* disclosed different chemical constitutions in each population and showed distinct activity against promastigote forms of *Leishmania amazonensis*, as well as toxicity

against peritoneal macrophages of BALB/c mice. Multivariate statistical analysis uncovered the influence of each constituent of the oils against *L. amazonensis* being 1,10-di-*epi*-cubenol, α -amorphene, *E*-caryophyllene, isopimara-7,15-diene, and β -elemene associated with the antileishmanial potential (Oliveira et al. 2019).

Projects of the BIOTA-FAPESP program have also been focusing on environmental aspects aiming at the development of more eco-friendly processes, e.g. optimizing separation and purification procedures during the isolation of natural products. Chemical studies generally involve the use of harmful organic solvents and reagents, which are not only toxic to people and to the environment, but also generate large amounts of waste. Thus, the Green Chemistry principles in association with chemometrics and experimental design statistics have been adopted both to investigate seasonal variations of the chemical composition of *Serjania marginata*, as well as they have been applied to HPLC-PDA methods used to standardize this plant extract before the biological assays (Zanatta 2021). Further examples include the replacement of organic solvents and optimization of extraction and chromatographic procedures aiming at the implementation of eco-friendly processes as a routine (Ferreira et al. 2016, Sutton et al. 2018).

The use of modern metabolomics approach to evaluate chemical and ecological interactions, understand biodiversity traits, compare different plant matrices and sources, and search for potentially innovative targets in complex extracts is an asset for the field of natural products chemistry. The possibility to use the obtained data in combination with modern computational tools, as well as with other *omics* technologies, is of great importance to expand the information retrieved in these analyses, in addition to making it possible to search for tendencies in specific groups, or to accelerate the search and development of new drugs or valuable compounds, for instance. However, the dereplication and annotation of natural products in complex matrices rely mainly on the available spectral libraries. These are far from containing all the spectral information of the natural products characterized to date, being the annotation still considered a bottleneck in metabolomic studies. Despite the noteworthy advances in the dereplication and annotation of natural products, the classical phytochemical methodologies are still crucial to fully characterize new molecules and determine their biological activities. Therefore, phytochemistry and the dereplication of metabolites are complementary approaches in the natural products field, each with pros and cons that must be considered, particularly in bioprospecting projects.

In summary, the BIOTA-FAPESP program has greatly provided researchers of São Paulo State with the possibility to use state-of-the-art analytical techniques and instrumentation, leveraging cutting-edge research and technological advances related not only to biodiversity products, but also contributing to the valuation of ecosystem services and biodiversity protection. Such a scenario evidences the remarkable advances the Natural Products area has experienced through the years (Figure 5) and represents a strong stimulus for innovative research and attraction of young talented scientists, which renders the formation of highly qualified human resources to tackle the upcoming challenges regarding the exploration of biodiversity in a sustainable manner with positive social, economic, and environmental impacts.

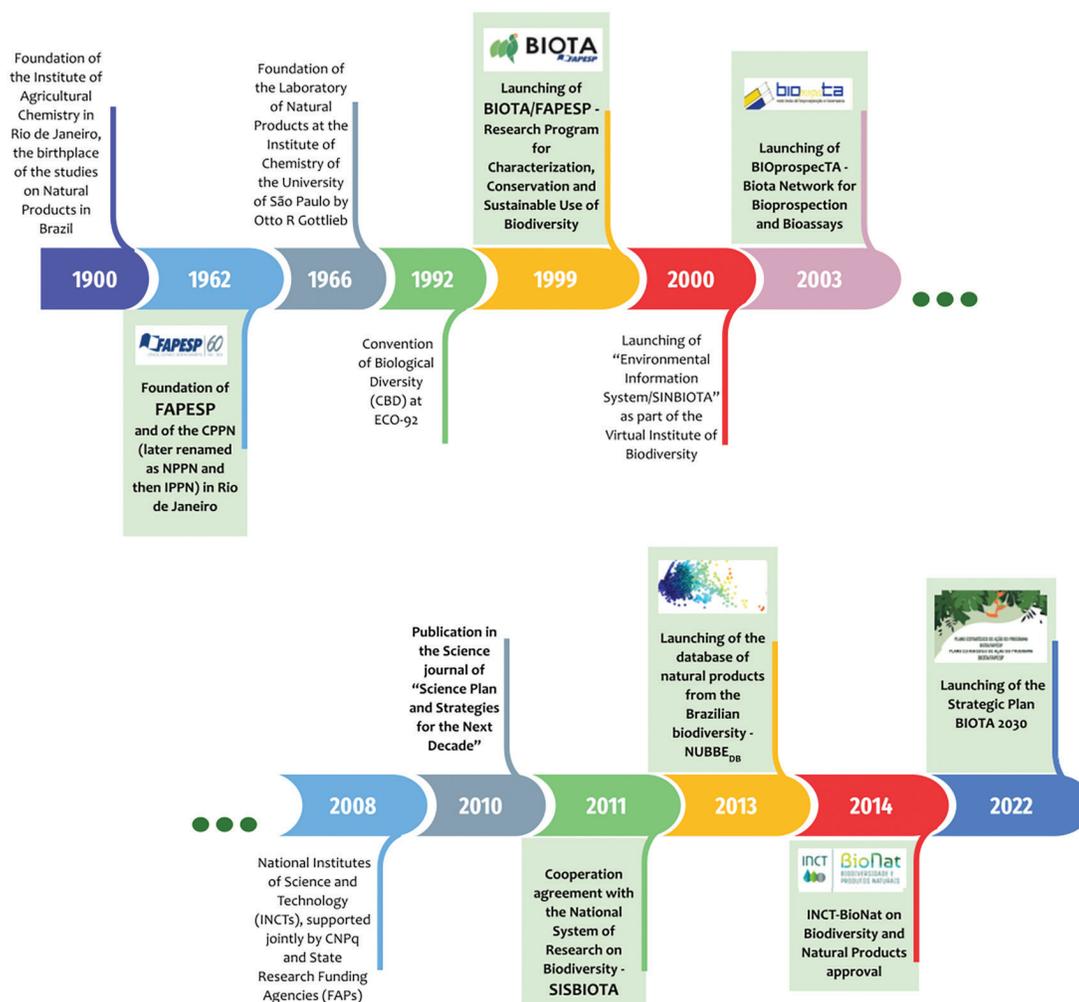


Figure 5. Timeline highlighting the main milestones of establishment and development of the Natural Products research area in Brazil.

Trends and Perspectives for Natural Products Research

The strong support of FAPESP and the high-quality results since BIOTA-FAPESP launching has stimulated similar initiatives from federal research funding agencies. For instance, the SISBIOTA Program, supported by CNPq in partnership with several State research funding agencies, was created in 2010. Such initiative strengthened collaborative research among several natural products research groups throughout Brazil, contributing to optimize resources and equipments uses. Later on, the launching of the National Institutes for Science and Technology (INCT) by CNPq, also in partnership with State research funding agencies, created new opportunities for wide scope research consortiums, which has played an important role in the establishment of research networks and improvement in human resources formation. Therefore, it is highly desirable that BIOTA-FAPESP further inspires novel initiatives that will contribute to better knowledge on natural resources and so many positive unfoldings all over Brazil.

Such knowledge is expected to gather competencies that might lead to significant improvements in the sustainable exploration of biodiversity, adding value to bioproducts and representing a real advance towards a fair benefit sharing, especially to populations that detain traditional knowledge on medicinal plants, alongside biodiversity protection initiatives. Additional environmental concerns might also be

increasingly addressed through the development of greener approaches in the discovery of natural products, especially those associated with the replacement of organic solvents and other consumables for chromatography as well as extraction protocols.

Data compilation, systematization, and sharing represent also crucial steps for solid advancements regarding the future of natural products research. The organization and curation of the Nuclei of Bioassays, Ecophysiology and Biosynthesis of Natural Products Database (NUBBE_{DB}), a database of natural products from the Brazilian biodiversity released in 2013, has continuously been improved to provide data of 2000+ compounds from plants and other natural sources, in addition to semi-synthetic compounds. Its contents on chemical, biological, pharmacological, and geographical coordinates of collection sites make this database a valuable asset for a deeper understanding of the natural products explored in the Brazilian territory. A collaboration with ACD/Labs also enables the user to access simulated NMR spectroscopic data from the curated compounds, being particularly useful for the characterization of isolated compounds. All NuBBE_{DB} features are freely accessible online (Valli et al. 2013, Pilon et al. 2017). A collaboration between São Paulo State University's Institute of Chemistry (IQ-UNESP) at Araraquara, Brazil, and the Chemical Abstracts Service (CAS), a division of the American Chemical Society

(ACS), will provide a huge expansion to compile more than 54,000 natural products and derivatives related to Brazilian biodiversity in a more complete database containing systematized information on their occurrence and chemical structure, as well as a list of published articles. In this context, NuBBE_{DB} major goals include assisting researchers and companies in the development of different fields of Brazilian science, technological development of biodiversity products with increased added value, strengthening bioeconomy and optimizing public policies.

One of the problems in using Brazilian medicinal plants is the lack of information about the safety and efficacy of the phyto-preparations. The BIOTA-FAPESP program also invested in projects dealing with the standardization of herbal products, since it requires a lot of cautiousness and strict adherence to adequate guidelines indicated by agencies such as the International Conference on Harmonization (ICH) (ICH, 2021) and the Brazilian National Health Surveillance Agency (ANVISA) (Anvisa, 2017). The chemical composition and the efficacy of a herbal medicine are strongly affected by variations among species, adulteration, environmental factors, extraction and the phyto-preparation method (Salem et al. 2020, Yuliana et al. 2011). The combination of analytical methods, metabolomic techniques and multivariate statistical analyses now plays an important role in standardization procedures.

Brazil is a megadiverse country regarding its biological resources, and the diversity of biomes makes Brazilian biodiversity unique. Several policies have been implemented to preserve and study these biomes, and the BIOTA-FAPESP program represents a paradigm shift for having undoubtedly contributed to a better understanding of nature's hidden secrets, in addition to stimulating the nucleation of several research groups all over Brazil. These past two decades showed that the studies regarding Brazilian flora in the BIOTA-FAPESP projects benefited from the interdisciplinary focus, which provided robust and rich results, also resulted from dozens of international collaborations established. These collaborations led to a marked increase in the impact of the scientific research produced in Brazil in this area. This is a very encouraging scenario, and leads us to imagine what the next 20 years hold for the researchers in the BIOTA-FAPESP program. Preservation policies, investment in science and technology, and collaboration with the private sector for value-added bioproducts are some of the challenges to be considered in this next chapter of the BIOTA-FAPESP history.

Supplementary Material

The following online material is available for this article:

Supplementary material 1 – Literature survey of the BIOTA proposals and published articles within the plant scope. **Sheet 1:** Information retrieved from the FAPESP Virtual Library in January 4th (2022) regarding all the proposals in the BIOTA-FAPESP program. **Sheet 2:** Information retrieved from the FAPESP Virtual Library in January 4th (2022) regarding all the proposals in the BIOTA-FAPESP program – Filtered to keep only proposals involving plant species, regardless of the field of study. **Sheet 3:** Articles linked to the proposals involving the study of plant species, specifically from the Chemistry and Pharmacy fields of knowledge. **Sheet 4:** Information retrieved from the FAPESP Virtual Library in January 4th (2022) regarding all the proposals in the BIOTA-FAPESP program – Filtered to keep only

proposals involving BIOPROSPECTION of plant species. **Sheet 5:** Information retrieved from the FAPESP Virtual Library in January 4th (2022) regarding all the proposals in the BIOTA-FAPESP program (organized by time of submission) – Filtered to keep only proposals involving BIOPROSPECTION of PLANT SPECIES.

Supplementary material 2 – BIOTA Projects on Plants Bioprospection.

Associate Editor

Carlos Joly

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

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