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Twenty-five years of natural products research in NuBBE

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The richness of Brazilian biodiversity translates into a valuable collection of molecules with biological properties that range from ecological functions to pharmacological properties. For over 25 years, the Nucleus of Bioassays, Biosynthesis, and Ecophysiology of Natural Products (NuBBE) has conducted extensive investigations into the chemical entities of numerous plant and microorganism species, resulting in the discovery of over a thousand natural compounds spanning various chemical classes (such as shikimate derivatives, phenylpropanoids, terpenoids, alkaloids, and peptides). The research goals within the natural products field encompass phytochemical studies, investigations of endophytic fungi and marine organisms, biosynthetic studies, medicinal chemistry, and the development of innovative methodologies. This comprehensive review article aims to offer valuable insights into the multifaceted research endeavors conducted in NuBBE. In this way, accomplishments, perspectives, and opportunities for advancing natural products research in Brazil are highlighted, seeking to inspire and motivate other research groups in the field of natural products–especially those located in emerging countries with rich biodiversity.

KEYWORDS

Brazilian biodiversity, isolation, bioactivity, methodologies, biosynthesis, peptides, computational tools

Introduction

Natural products are compounds biosynthesized from different groups of living organisms, such as plants, fungi, and bacteria (Sorokina and Steinbeck, 2020). They have played a critical role in the progress of human civilization and society as we know it today. Throughout history, natural medicines from diverse organisms have been utilized for the treatment of various diseases and conditions. The therapeutic potential of natural products continues to inspire the development of new drugs and therapies until today (Newman and Cragg, 2020). Paclitaxel, from the Pacific Yew tree (*Taxus brevifolia*) (Wani et al., 1971; Croteau et al., 2006), statins derived from several *Penicillium* species (Endo, 2008), vincristine from the Madagascar periwinkle plant (*Catharanthus roseus*) (Noble, 1990), and angiotensin-converting enzyme (ACE) inhibitors, such as enalapril and lisinopril isolated from compounds found in the venom of the Brazilian pit viper (*Bothrops jararaca*) (Oliveira et al., 2022), are just a few examples among many others.

Brazil, with its vast and diverse territory spanning half of South America, is recognized as one of the world's most important biodiversity hotspots. It is home to approximately 20% of the total species on the planet, showcasing a remarkable array of fauna and flora. The country encompasses six biomes, each characterized by unique ecological features and species composition: the Amazon Forest, the largest tropical rainforest in the world; the Atlantic Forest, which stretches along the entire coastal region; the Cerrado, renowned for its rich plant species diversity; the Pantanal, recognized by UNESCO as a Biosphere Reserve and World Heritage Site; the Caatinga, a unique semi-arid biome rich in drought-resistant plant species, primarily located in northeastern Brazil; and the Pampas, exclusive to the southern region of the country (MMA, 2023).

The great Brazilian biodiversity has stimulated the establishment of numerous research groups across the country dedicated to studying and harnessing the potential of natural resources in a sustainable way. One such group is the Nucleus of Bioassays, Biosynthesis, and Ecophysiology of Natural Products (NuBBE), located at the São Paulo State University (UNESP). Since its inception in 1998, NuBBE has been dedicated to studying natural products from Brazilian biodiversity, particularly focusing on plant species from the Cerrado and Atlantic Forest (Valli et al., 2018; Silva et al., 2022; Vieira et al., 2023b).

Throughout its 25 years of history, NuBBE has provided an infrastructure and scientific background for the education and training of approximately 400 individuals, including undergraduate and graduate students, as well as post-doctoral researchers. Their work has not only contributed to NuBBE's research endeavors but has also had a broader impact, as the knowledge and expertise of several of these people played a key role in the consolidation of other research groups and the expansion of the natural products community across different regions of Brazil.

While the group initially focused mainly on phytochemical and biosynthesis of studies of plants species from Fabaceae, Pipearaceae, Celastraceae, its research scope rapidly expanded to include investigations of endophytic fungi from plants and marine organisms, medicinal chemistry and semi-synthesis, and the optimization and development of novel methodologies, including integrating computational approaches and simulations of complex natural matrices–topics that will be further discussed in details in the following sessions. Also, as a result of their investigations, NuBBE established the first Brazilian Natural Products database, which is a comprehensive resource encompassing the rich Brazilian biodiversity. This database includes information about compounds, predicted spectral data, biological activities, locations, and various medicinal chemistry features.

Over these past two decades, the group has contributed to the chemical study of 380 plant and 41 microorganism species, which resulted in the isolation of 1,283 natural compounds from diverse chemical classes (Figure 1; Figure 2). Efforts have been made towards an extensive investigation regarding their bioactivities, including but not limited to antioxidant, antitumoral, anticancer, antifungal, and antimalarial properties. Research in plant ecophysiology also allowed the identification of natural biomarkers signaling both biotic and abiotic influences on plant biosynthesis. These valuable findings provided only a glimpse into the vast potential of natural products from Brazilian biodiversity and highlighted that there is much more to be explored in the country.

In this review article, we aim to shed light on the multifaceted research conducted by the group over the past 25 years. These efforts share a common goal: the sustainable and rational use and exploration of Brazilian biodiversity. In this way, we hope that it can inspire and encourage other research groups in the field of natural products in Brazil and worldwide, particularly those from emerging countries with rich biodiversity and still in consolidation. Moreover, from the lessons learned, we describe perspectives, gaps, and opportunities for the natural products research field in Brazil.

Natural products research in nubbe

Natural products from plants

The NuBBE research group has made significant contributions to phytochemical studies of plants, uncovering compounds with potent bioactivities (Figure 3). Some examples will be highlighted in this session.

Natural products with cytotoxic activity were the focus of several studies conducted in the group. During a study conducted with *Casearia rupestris*, four new clerodane diterpenes, namely casearupestrins A-D (**1**–**4**), were isolated. Casearupestrin A exhibited remarkable cytotoxicity against MDA/MB-435 (human melanoma) and SF-295 (human glioblastoma) cells, with a half maximal inhibitory concentration (IC_{50}) value of 0.36 μM, surpassing the efficacy of the standard drug doxorubicin (IC_{50} = 0.83 μM and 0.40 μM, respectively). Interestingly, the acetylation of two compounds [2,7-di-*O*-acetylcasearupestrin A (**5**) and 2,6-di-*O*-acetylcasearupestrin D (**6**)] led to a significant decrease of the bioactivity, indicating the importance of the C-2 OH group for the activity (Vieira-Júnior et al., 2011). *Casearia sylvestris* was also investigated, leading to the isolation of another new clerodane diterpene, casearin X (**7**), along with the known compounds casearins B (**8**), D (**9**), L (**10**), and O (**11**), and caseargrewiin F (**12**). Notably, caseargrewiin F exhibited the most significant cytotoxicity against four human tumor cell lines, namely MOLT-4 (leukemia), MDA-MB-435 (melanoma), HCT-8 (colon), and SF-295

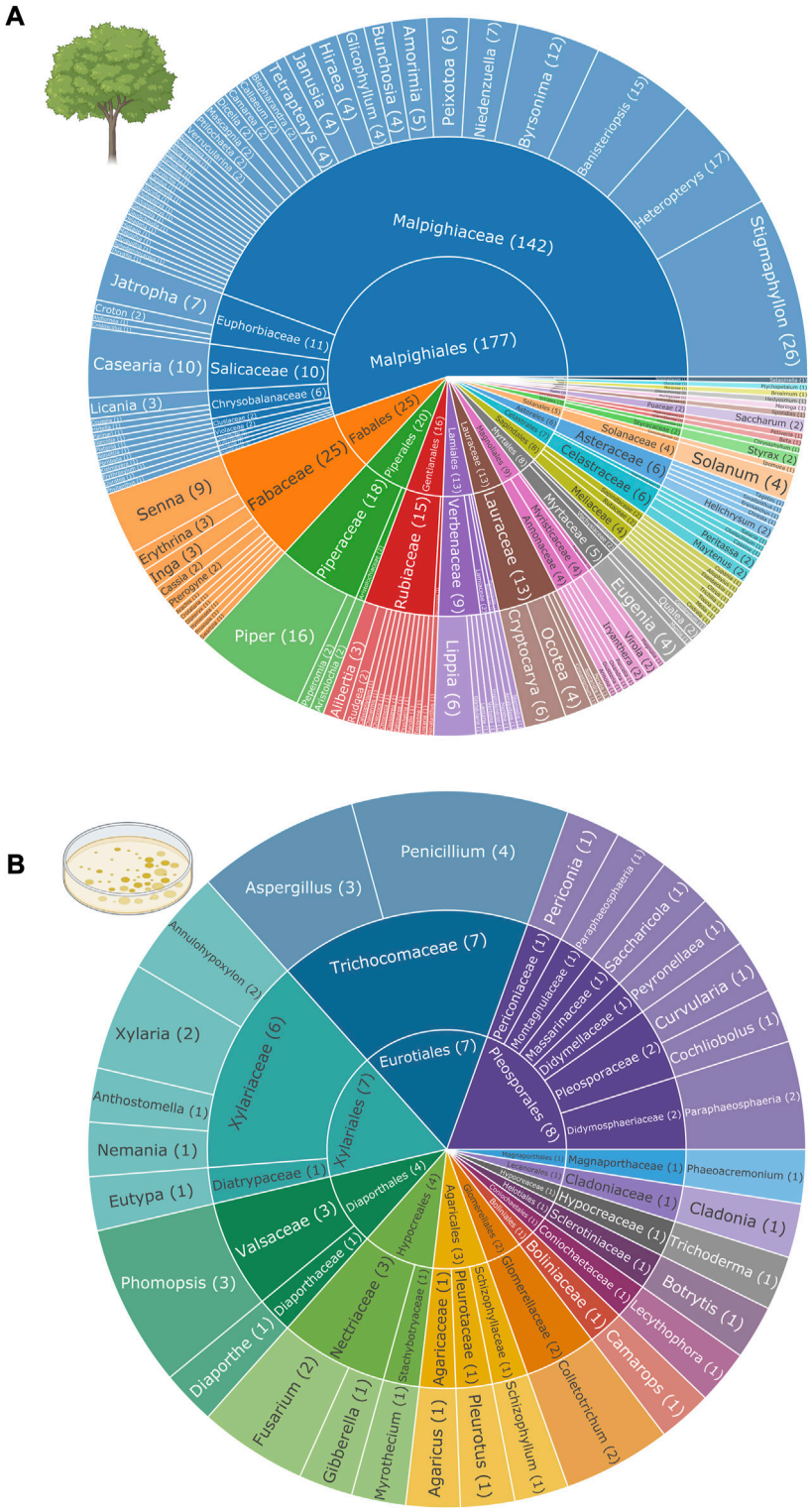


FIGURE 1
Overall distribution of plant **(A)** and microorganisms **(B)** species investigated over the past 25 years in NuBBE. Numbers in parenthesis are referred to the number of species studied in each taxonomic level (order, family, and genus). Icons were obtained from BioRender.com. Figure in full resolution is also provided as Supplementary Figure S1.

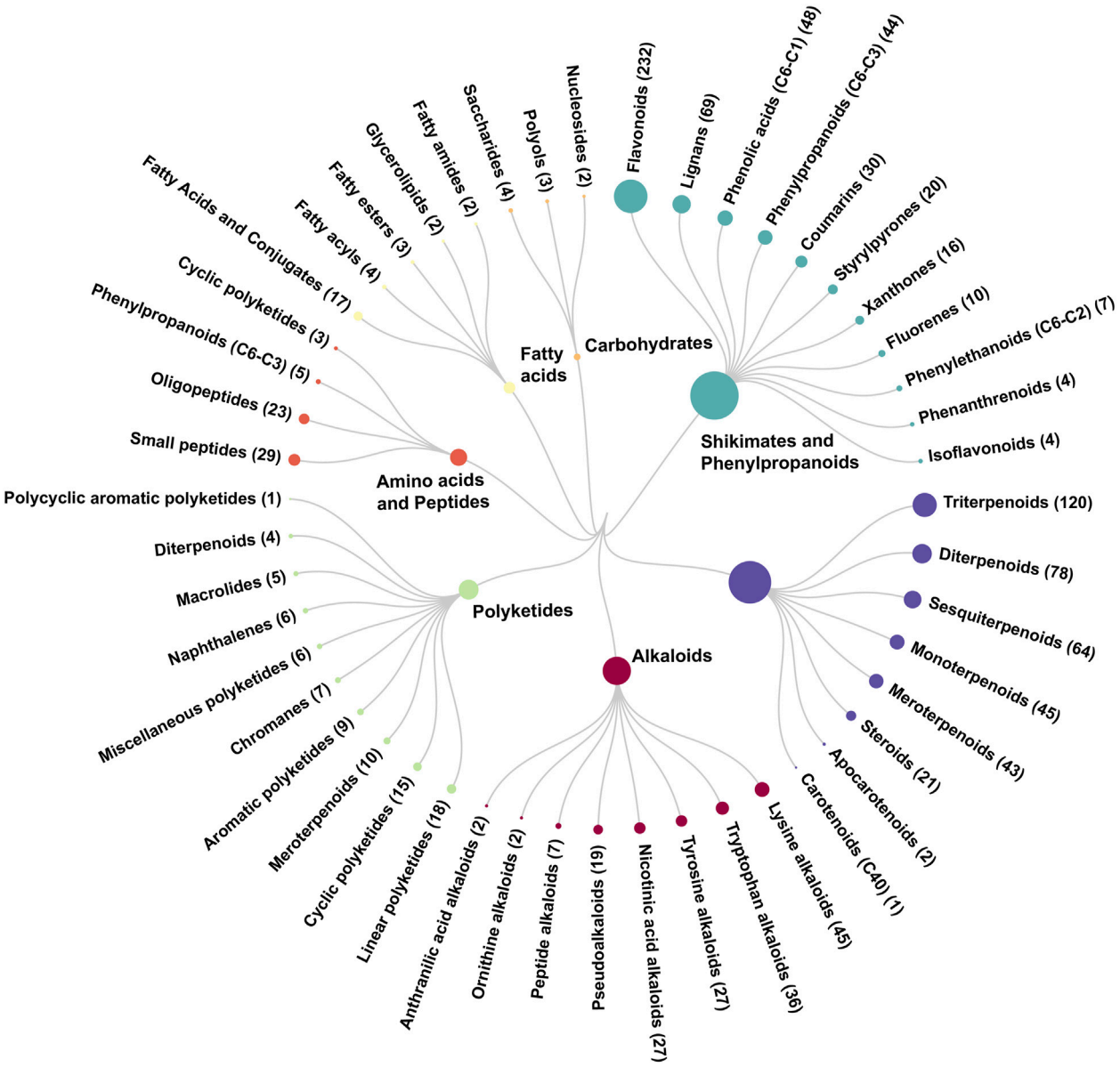
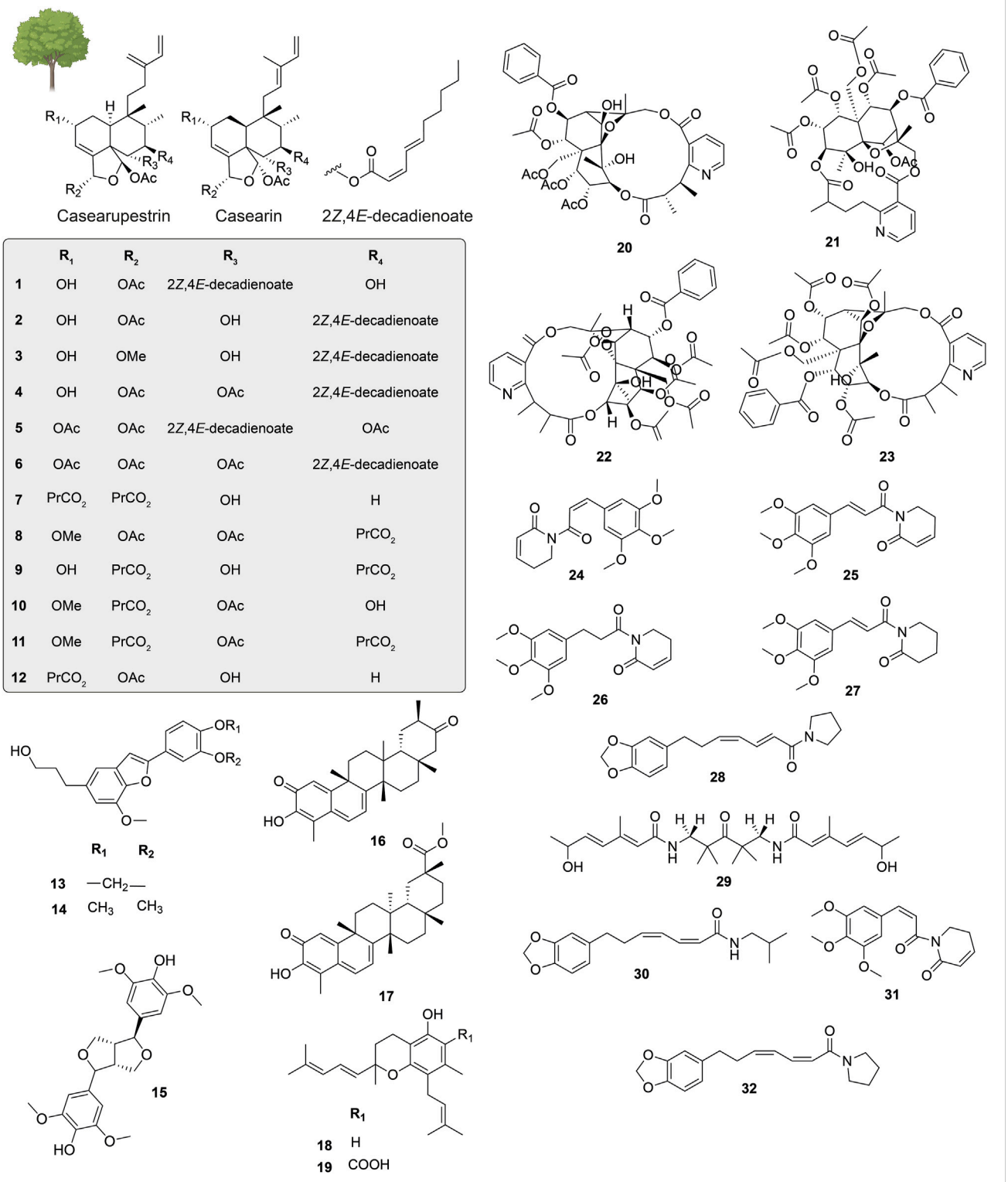


FIGURE 2
Overall distribution of the classes of compounds isolated over the past 25 years in NuBBE. Classification was carried out with NPClassifier comprehending carbohydrates, fatty acids, amino acids and peptides, polyketides, alkaloids, terpenoids and shikimic acid and phenylpropanoids derivatives (Kim et al., 2021). Numbers in parenthesis are referred to the number of compounds isolated in each superclass.

(glioblastoma), with IC_{50} values below 0.2 μM. All the isolated diterpenes showed lower activity against normal human cells compared to cancer cell lines, suggesting a potential selective action (dos Santos et al., 2010).

Lignans were also investigated for cytotoxic activity in a study conducted by Teles et al. (2005). In this study, the benzofuran lignans egonol (**13**) and homoegonol (**14**), in addition to the furofuran lignan syringaresinol (**15**), were isolated from *Styrax camporum*. These compounds presented cytotoxic effects against Hep-2 (larynx epidermoid carcinoma), HeLa (cervix carcinoma), and C6 (rat glioma) cell lines. Notably, egonol demonstrated significant activity against C6 (IC_{50} = 3.2 μg/mL) and Hep-2 (IC_{50} = 3.6 μg/mL) cell lines, while homoegonol showed potency against C6 (IC_{50} = 4.9 μg/mL) and HeLa (IC_{50} = 5.3 μg/mL) cells (Teles et al., 2005).

As part of a bioprospecting program aimed at the discovery of antiprotozoal agents from the Brazilian flora, different classes of secondary metabolites showed interesting trypanoside and leishmanicidal activities. In a study conducted with *Maytenus ilicifolia*, the quinonemethide triterpene maytenin (**16**) and pristimerin (**17**) were isolated and presented potent leishmanicidal activity against *Leishmania amazonensis* and *Leishmania chagasi*, with IC_{50} values below 0.88 nM. In addition, these compounds also displayed potent activity against *Trypanosoma cruzi* (IC_{50} < 0.3 nM) (Dos Santos et al., 2013). Furthermore, phenolic derivatives from *Peperomia obtusifolia* also demonstrated trypanocidal activity. Among the compounds isolated,



	R_1	R_2	R_3	R_4
1	OH	OAc	2Z,4E-decadienoate	OH
2	OH	OAc	OH	2Z,4E-decadienoate
3	OH	OMe	OH	2Z,4E-decadienoate
4	OH	OAc	OAc	2Z,4E-decadienoate
5	OAc	OAc	2Z,4E-decadienoate	OAc
6	OAc	OAc	OAc	2Z,4E-decadienoate
7	$PrCO_2$	$PrCO_2$	OH	H
8	OMe	OAc	OAc	$PrCO_2$
9	OH	$PrCO_2$	OH	$PrCO_2$
10	OMe	$PrCO_2$	OAc	OH
11	OMe	$PrCO_2$	OAc	$PrCO_2$
12	$PrCO_2$	OAc	OH	H

FIGURE 3
Examples of secondary metabolites isolated from plant species in studies conducted in NuBBE research group. Icons were obtained from BioRender.com.

peperobtusin A (**18**) and 3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(2″-methyl-2″-butenyl)-2-(4′-methyl-1′,3′-pentadienyl)-2H-1-benzopyran-6-carboxylic acid (**19**) exhibited the most potent activities, with IC_{50} values of 3.1 μM and 27.0 μM, respectively (da Silva Mota et al., 2009).

Alkaloids also stand out for presenting antiprotozoal activities. For instance, a study conducted with *M. ilicifolia* species resulted in the isolation of two new sesquiterpene pyridine alkaloids, ilicifoliunines A and B (**20** and **21**), along with the known

alkaloids aquifoliunine EI (**22**) and mayteine (**23**). Aquifoliunine EI displayed potent activity against *L. chagasi* and *T. cruzi*, with IC_{50} values of 1.4 and 41.9 μM, respectively. Aquifoliunine EI also exhibited low cytotoxicity against murine peritoneal macrophages. The alkaloid ilicifoliunine A demonstrated potent antitrypanosomal activity, with an IC_{50} value of 27.7 μM (Santos et al., 2012). Additionally, from *Piper tuberculatum*, eight piperamides were isolated and evaluated against the epimastigote forms of *T. cruzi*. The natural isomers of piplartine (**24** and **25**) exhibited potent activity against *T. cruzi*, with the *Z* isomer being twice as active as the *E* isomer (*Z*, IC_{50} = 21.0 μM). The natural compound 8,9-dihydropiplartine (**26**), with one less double-bound, was slightly less active than isomers piplartines, whereas the synthetic derivative tetrahydropiplartine (**27**), with two double bonds less, was even less active (Cotinguiba et al., 2009).

Efforts were also made towards investigating antifungal activities of various natural compounds, including amides bearing isobutyl, pyrrolidine, dihydropyridone, and piperidine moieties. Several studies highlighted the antifungal potential of compounds isolated from several Piperaceae species (Alécio et al., 1998; Navickiene et al., 2000; Vasques da Silva et al., 2002). For instance, a bioactivity-guided fractionation o *Piper arboreum*, *Piper tuberculatum* and *Piper hispidum* led to the isolation of five new amides, *N*-[10-(13,14-methylenedioxyphenyl-7(*E*),9(*Z*)-pentadienoyl]-pyrrolidine (**28**), arboreumine (**29**), (3*Z*,5*Z*)-*N*-isobutyl-8-(3′,4′-methylenedioxyphenyl)-heptadienamide (**30**), 8(*Z*)-*N*-(12,13,14-trimethoxycinnamoyl)-Δ3-pyridin-2-one (**31**), and *N*-[7-(3′,4′-methylenedioxyphenyl)-2(*Z*),4(*Z*)-heptadienoyl] pyrrolidine (**32**), together with other 14 known compounds. Most of these compounds exhibited antifungal activity against *Cladosporium sphaerospermum*, while the compounds isolated form *P. tuberculatum* were also active against the fungi *Cladosporium cladosporioides*. Furthermore, polysulphides from *Petiveria alliacea* (Benevides et al., 2001) and nor-lignans from *Styrax ferrugineus* (Pauletti et al., 2000) also showed potent antifungal activities.

Over the course of these 25 years, several studies conducted in the group extended beyond cytotoxic, antiprotozoal, and antifungal activities. Nitropropanoyl glycosides isolated from *Heteropterys umbellata* showed larvicidal activity against the *Aedes aegypti* Rockefeller strain, the vector of several neglected tropical diseases (Mannochio-Russo et al., 2023). Moreover, benzylisoquinoline alkaloids obtained from the leaves of *Ocotea paranapiacabensis* were found to inhibit the formation of advanced glycation end products, being these compounds also active in the anti-aging assay using a reconstructed *in vitro* human skin model, being promising compounds to be used in skin care products (Freitas et al., 2020). In addition, the erythrinian alkaloids isolated from *Erythrina mulungu*, a plant with ethnopharmacological importance and part of a list of medicinal plants elaborated by the Brazilian Ministry of Health (da Saúde, 2023), showed anxiolytic effects when investigated *in vivo* by the elevated T-maze test (Flausino et al., 2007). In another study, four new clerodane diterpenes obtained from *Croton floribundus* (Euphorbiaceae) exhibited inhibitory activity of nitrite production against murine macrophage-like cell lines (RAW 264), suggesting potential anti-inflammatory properties (Queiroz et al., 2020).

Several Brazilian edible fruits were also investigated in order to understand the health effects of compounds ingested through the diet, such as *Garcinia brasiliensis* (“bacupari”), *Spondias tuberosa* (“umbu”), and *Eugenia jambolana* (“jambolão”) (Arwa et al., 2015; Zeraik et al., 2016; Dametto et al., 2017). In addition, compounds of high added value were also investigated in non-edible wastes of *Platonia insignis* (“bacuri”) and *Talisia esculenta* (“pitomba”) (Ribeiro et al., 2021; Felix Alves et al., 2023). These commonly discarded materials contain high amounts of compounds such as flavonoids and biflavonoids, which are important standards for natural products research.

Cyclic peptides from plants

Naturally occurring bioactive peptides were the primary source of peptide drugs in the last century (Zhang and Chen, 2022). Compared to the major families of small molecules, peptides stand out for being synthesizable macromolecules that can be obtained as copies of their naturally occurring pattern or even in the form of various analogs. Structurally, they can be arranged in linear and cyclized backbones. Cyclic peptides have been shown to have higher biological activity compared to their linear counterparts due to conformational rigidity (Horton et al., 2002; Joo, 2012). In addition, these compounds have interesting biochemical and therapeutic properties for pharmaceutical applications, making them excellent group lead compounds (Joo, 2012; Zhang and Chen, 2022).

Orbitides (Craik et al., 2018) and cyclotides (Craik et al., 1999) are plant cyclic peptides with distinct structural arrangements. In 2009, after an exhaustive search in the main Natural Products databases, researchers noticed the scarcity of studies focusing on these cyclic peptides from Brazilian plants. In light of this, NuBBE has been dedicating efforts in studying these two classes of ribosomally synthesized cyclic plant peptides from Euphorbiaceae and Violaceae species.

The Euphorbiaceae family is described as a source of bioactive orbitides (Sabandar et al., 2013; Ramalho et al., 2018a). These peptides are a class of natural ribosomally synthesized cyclic peptides, with head-tail cyclization, ranging from 5 to 12 amino acids. More than 20 orbitides have been reported in the literature from *Jatropha* L. and *Croton* L. genera (Ramalho et al., 2018a), and two of them have been described in NuBBE: the octapeptides jatrophidin (**33**) and ribifolin (**34**) (Altei et al., 2014; Pinto et al., 2015) (Figure 4A).

Jatrophidin was isolated from the latex of *Jatropha curcas* L., and its structure was determined as *c* (PGLLNLWG) (**33**) after 2D NMR spectroscopic analysis, quantitative amino acid analysis, application of Marfey’s method for determining the absolute configuration of its amino acid residues, and conformational studies using Molecular Dynamics/Simulated Annealing (MD/SA) (Altei et al., 2014). Jatrophidin was tested for several biological activities and showed moderate protease inhibition activity when compared with pepstatin A. However, this compound was found to be inactive in antimalarial, antifungal, and antioxidant assays (Altei et al., 2014).

On the other hand, ribifolin (**34**), was isolated from the aerial parts of *Jatropha ribifolia* (Pohl) Baill (Pinto et al., 2015), and its sequence was determined as *c* (SGLIGLII) using the aforementioned techniques (Altei et al., 2014), and Raman optical activity measurements and calculations. Pinto and coworkers also described the synthesis of ribifolin and its linear form by solid-phase peptide synthesis. Both structures were evaluated for their

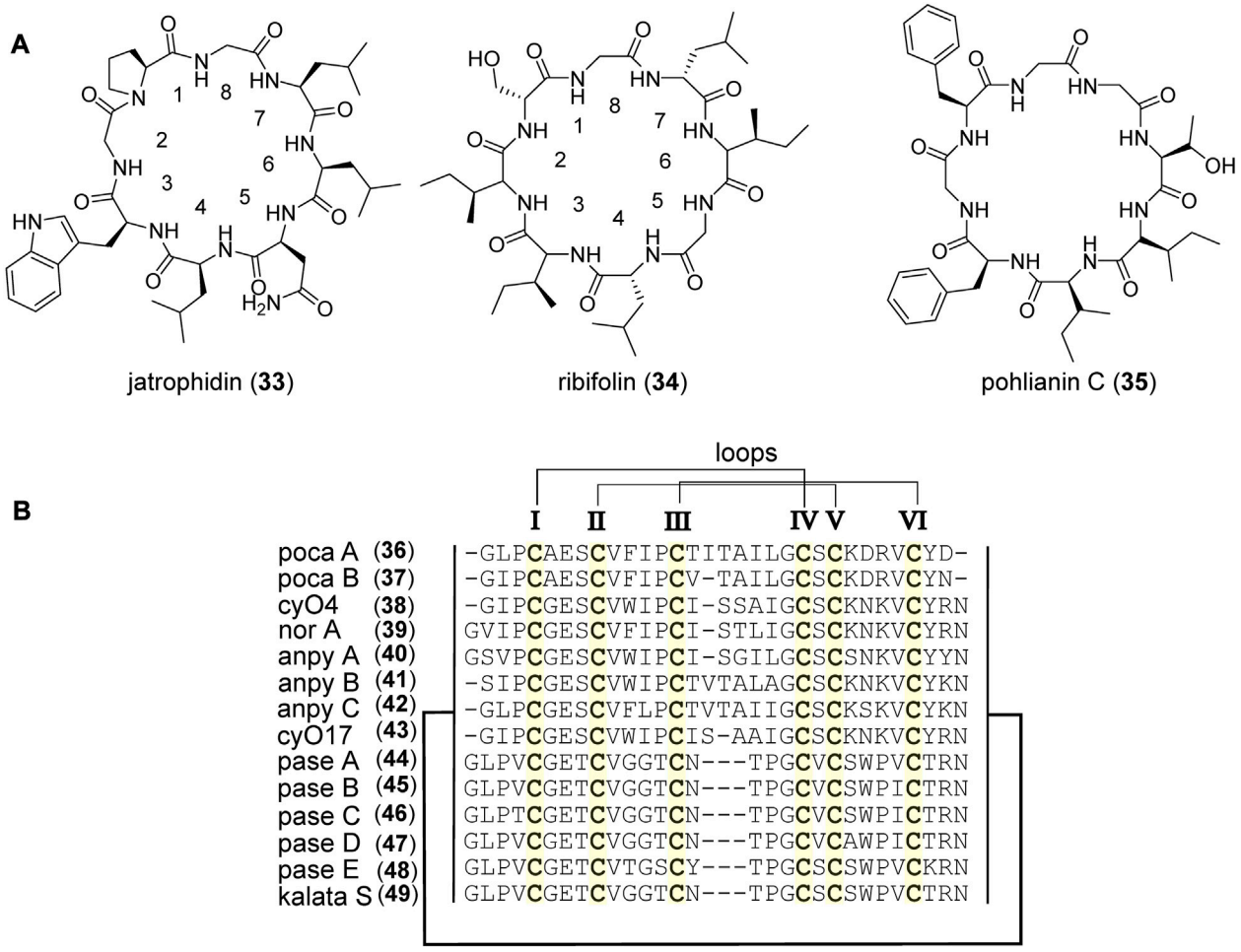


FIGURE 4
Summary of the isolated and sequenced orbitides and cyclotides. **(A)** Ribifolin and jatrophidin were isolated from *Jatropha ribifolia* and *Jatropha curcas*, respectively. **(B)** Fourteen cyclotides isolated from *Pombalia calceolaria*, *Palicourea sessilis*, *Noisettia orchidiflora*, and *Anchietea pyrifolia*.

antiplasmodial and cytotoxic activities. Ribifolin demonstrated moderate antiplasmodial activity (IC_{50} = 42 μM), while the linear analog was inactive (IC_{50} = 519 μM) (Pinto et al., 2015).

To provide additional structural information, a study describing the X-ray crystallography of jatrophidin, ribifolin, and pohlianin C (**35**) (another *Jatropha* orbitide previously reported) was performed (Ramalho et al., 2018b). Racemic crystallography can provide powerful complementary structural information or be an alternative to traditional crystallography on enantiopure samples. Thus, the D-enantiomers of these peptides were also synthesized and crystallized, in mixtures of equal ratios with L-isomers, for analysis. The high-resolution structure of ribifolin (0.99 Å), pohlianin C (1.20 Å), and jatrophidin (1.03 Å) was obtained, and the permeability of these orbitides and their methylated analogs in the membrane and in cells was investigated in a parallel artificial membrane permeability assay (PAMPA) and a Caco-2 cell assay. [NMe-ILG]-ribifolin showed the highestpermeability through the PAMPA assay (Ramalho et al., 2018b). The effects of the synthetic pohlianin A-C, ribifolin, and their linear analogs on the migration of neonatal human foreskin fibroblasts *in vitro* assays were also assessed. The results showed that pohlianin B and linear ribifolin have the potential to induce fibroblast migration and collagen deposition, thus contributing to accelerating the processes of wound healing and tissue repair (Ramalho et al., 2019).

Cyclotides comprise another class of cyclic peptides, usually composed of 28–37 amino acids, stabilized by three disulfide bonds forming a cyclic cystine knot (Craik et al., 1999). They are ubiquitous in the Violaceae family, with sparse reports in Rubiaceae, Cucurbitaceae, Solanaceae, and Fabaceae (Gerlach et al., 2013). Two novel cyclotides, poca A and B (**36**–**37**), and the previously reported cycloviolacin 04 (Cy04) (**38**), were described from the roots of *Pombalia calceolaria* (Violaceae) (Pinto et al., 2018). After reduction and alkylation reactions, as well as enzymatic digestion of the isolated peptides, these peptides were sequenced by MALDI-TOF/TOF mass spectrometry. An interesting contribution described in this study was the MALDI imaging experiment involving cyclotides, which provides powerful complementary information to traditional histology (Aichler and Walch, 2015). This approach demonstrated the presence of poca A-B and cyO4 in the phloem and cortical parenchyma regions of the roots. Moreover, these cyclotides were evaluated against human cancer cell lines MDA-MB-231 (metastatic breast cancer), leading to more than 80% of the cancer cell death at 20 μM, with IC_{50} values of 1.8, 2.7, and 9.8 μM, respectively, for poca A, poca B, and cyO4.

Additionally, in the wound-healing assay, cyO4 was in being able to inhibit cell migration (50%) at a subtoxic concentration (2 μM) (Pinto et al., 2018).

Other species from the Violaceae family were also studied regarding their cyclotide composition: *Noisettia orchidiflora* and *Anchietea pyrifolia*. An investigation conducted with the stems of *N. orchidiflora* resulted in a new cyclotide named nor A (**39**) (Bobey et al., 2018), while the roots of *A. pyrifolia* yielded three new cyclotides, anpy A–C (**40–42**), in addition to the known cycloviolacins O4 (cyO4, **38**) and O17 (cyO17, **43**), being the first report of cyclotides from the genus *Anchietea* (Fernández-Bobey et al., 2022). In both studies, the peptide sequences were determined using the same techniques previously described. The cyclotides anpy A–C, cyO4, and cyO17 were evaluated against three human cancer cell lines: colorectal carcinoma cells (HCT 116 and HCT 116 *TP53*$^{-/-}$) and breast adenocarcinoma (MCF 7), with IC_{50} values ranging between 0.8 and 7.3 μM CyO17 was the most potent cyclotide for the colorectal cancer cell lines, with IC_{50} 0.8 and 1.2 μM. Furthermore, the hemolytic activity of the four of these peptides was assessed, and anpy A was the most hemolytic with a concentration for inducing 50% of erythrocyte hemolysis (HD_{50}) of 22 μM (Fernández-Bobey et al., 2022).

In addition to cyclotides from Violaceae, cyclotides in the Rubiaceae family were also investigated for the species *Palicourea sessilis*. In this study conducted with leaves and stems from *P. sessilis*, five new cyclotides, pase A–E (**44–48**), as well as the known peptide kalata S (**49**). The sequences of these compounds were determined using MALDI-TOF/TOF and NMR experiments. These compounds exhibited high homology with the immunomodulatory cyclotide T20K. Due to their structural similarity, pase A–D were assayed for cell proliferation of human primary activated T lymphocytes and showed IC_{50} values for pase A (4.5 ± 2.65 μM), pase B (2.3 ± 1.39 μM), pase C (7.1 ± 3.56 μM), and pase D (1.6 ± 1.78 μM). The toxicity on other non-immune cells, including immune cells (PBMCs), red blood cells (RBCs), umbilical vein endothelial cells (HUVECs), and cancer (HT-29) cells. Pase cyclotides showed low cytotoxicity and no discernible hemolytic effects at the tested concentrations (Pinto et al., 2021).

To date, 28 cyclotides and 8 orbitides have been isolated from Brazilian plants. Among them, 14 cyclotides and two orbitides have been discovered through the efforts of NuBBE. Given that the majority of the plant species mentioned in this session are endemic to South America (https://powo.science.kew.org/), this represents a vast opportunity to expand the research on cyclic peptides, since most of these peptides reported to date are from other continents, such as Oceania, North America, Asia. For instance, from 81 species of Violaceae present in Brazilian territory, 29 of these are endemic, providing an exceptional source of novel cyclotides that can be explored.

Natural products from microorganisms

Endophytic fungi from plants

While plants have long been recognized as a rich source of natural products with medicinal properties, endophytic fungi from plants have gained increasing attention in the past decades as an equally promising and largely underexplored source of bioactive compounds. These microorganisms live within the plant tissues in a symbiotic relationship with the host and have evolved to produce bioactive compounds that can help them to survive and thrive in their unique ecological niche (Aly et al., 2011). Although plant fungal endophytes have been known for over a century, it was only in the past few decades that they received more attention due to their pharmaceutical and ecological significance (Gunatilaka, 2006). A remarkable example of its importance is the detection of paclitaxel (Taxol®), an important anti-cancer compound, in *Taxomyces andreanae*, an endophytic fungus of *Taxus brevifolia* (Stierle et al., 1993; Aly et al., 2010), which led to an increased interest in searching for other bioactive compounds produced by endophytic fungi. The studies under this topic conducted in the NuBBE research group described the isolation, characterization, and biological evaluation of endophytic fungi mainly isolated from the plant species *Alibertia macrophylla*, *Cassia spectabilis*, *Piper aduncum*, and *Senna spectabilis* (Figure 5).

Penicillium species isolated from *Albertia macrophylla* leaves were found to produce orcinol (**50**), cyclo-(L-Pro–L-Val) (**51**), uracil (**52**), in addition to the dihydroisocoumarins 4-hydroxymellein (**53**), 8-methoxymellein (**54**), 5-hydroxymellein (**55**), (*R*)-7-hydroxymellein (**56**), and (3*R*,4*R*)-4,7-dihydroxymellein (**57**) (Oliveira et al., 2009; Oliveira et al., 2011). Compounds **50**, **53**, **56**, and **57** presented antifungal activity against the phytopathogenic fungi *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*, while compounds **51**, **56**, and **57** were active in acetylcholinesterase (AChE) inhibitory activity *in vitro*.

Camarops species from *A. macrophylla* were also investigated for bioactive compounds, resulting in the isolation of five new eremophilane-type sesquiterpenes, which were isolated and characterized as xylarenones C–G (**58–62**). Among these, xylarenone C was the only compound with potent inhibitory activity against pepsin (IC_{50} = 0.288 μM) and subtilisin (IC_{50} = 0.462 μM) proteases, suggesting a negative correlation between protease inhibitory activity and hydroxyl groups on the aliphatic side chain of xylarenones D and E (de Oliveira et al., 2011). Xylarenones C, D, F, and G were additionally described as potent inhibitors of reactive oxygen species (ROS) produced by stimulated neutrophils, with IC_{50} ranging from 4.17 to 6.13 μM, values comparable to the standards quercetin and apocynin (Gubiani et al., 2014). These results suggest that these xylarenones have the potential to be used as anti-inflammatory and antioxidant agents. In addition, xylarenones C and D demonstrated cytotoxicity against leukemia (HL-60), melanoma (MDA/MB-435), colon (HCT-8), and glioblastoma (SF-295) human tumor lines (Gubiani et al., 2016).

Studies on *Phomopsis cassiae*, an endophyte isolated from the leaves of *C. spectabilis*, led to the identification of seven new compounds: ethyl 2,4-dihydroxy-5,6-dimethylbenzoate (**63**), phomopsilactone (**64**), two diastereoisomeric 3,9,12-trihydroxycalamenenes (**65**, **66**); 3,12-dihydroxycalamenene (**67**); 3,12-dihydroxycadalene (**68**), and 3,11,12-trihydroxycadalene (**69**). Compounds **63**, **64**, and **69** presented strong antifungal activity against *C. cladosporioides* and *C. sphaerospermum*, and compounds **63** and **64** showed cytotoxicity against human cervical tumor cell line (HeLa) *in vitro* (Silva et al., 2005; 2006).

Xylaria species isolated from *P. aduncum* were also the subject of studies in NuBBE. From these fungi, a new dihydroisocoumarin, (3*R*,4*R*)-3,4-dihydro-4,6-dihydroxy-3-methyl-1-oxo-1H-isochromene-5-carboxylic acid (**70**), was isolated, exhibiting moderate antifungal activity against *C.*

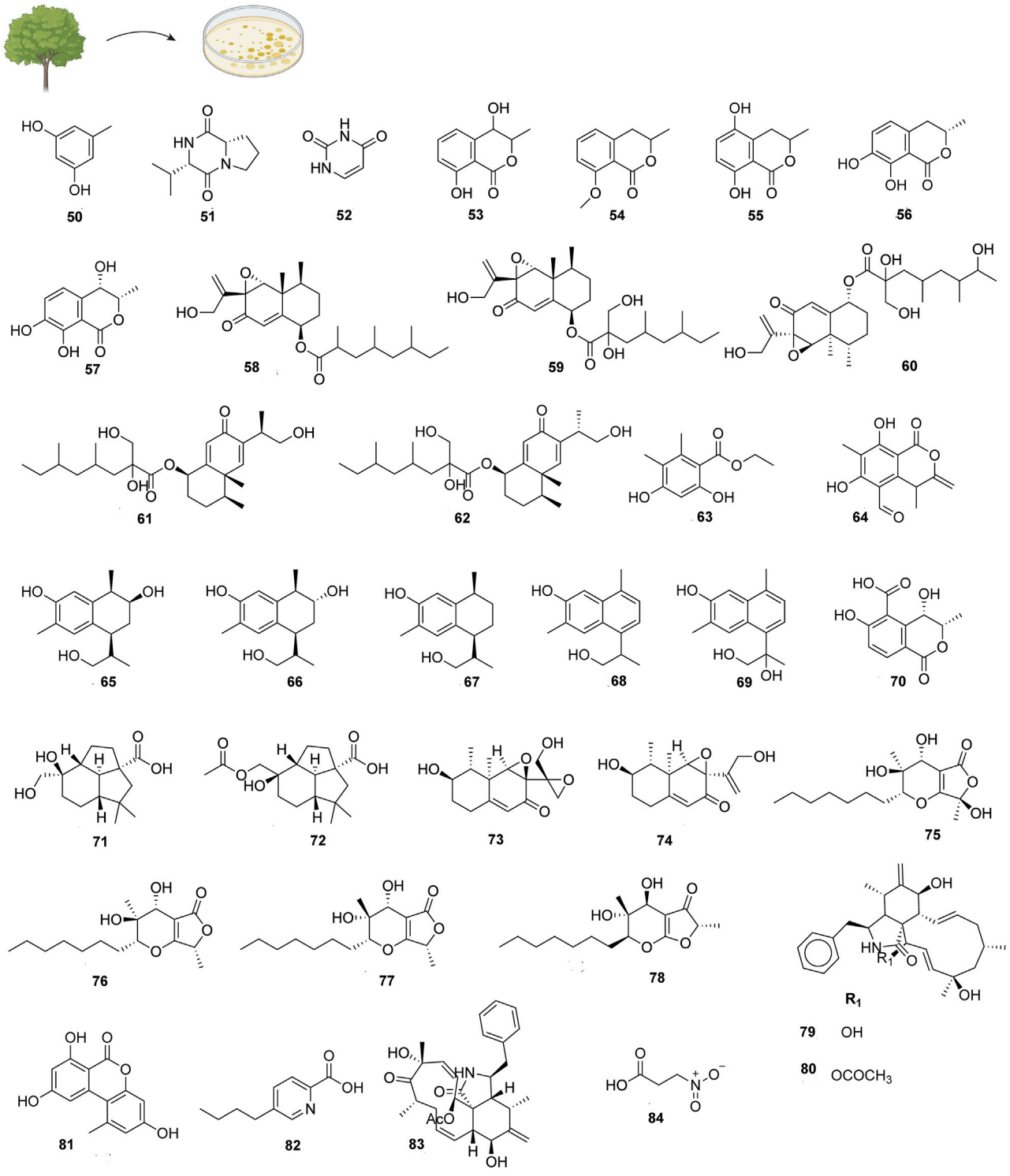


FIGURE 5
Examples of secondary metabolites isolated from plant endophytic fungi in studies conducted in NuBBE research group. Icons were obtained from BioRender.com.

cladosporioides and *C. sphaerospermum*, as well as moderate AChE inhibition (Oliveira et al., 2011). Furthermore, two new presilphiperfolane sesquiterpenes, 9,15-dihydroxy-presilphiperfolan-4-oic acid (**71**) and 15-acetoxy-9-hydroxy-presilphiperfolan-4-oic acid (**72**) were identified along with two known eremophilane sesquiterpenes, phaseolinone (**73**) and phomenone (**74**). Compound **73** exhibited moderate cytotoxicity in Chinese Hamster Ovary (CHO) cell line, while compound **74** was identified as a potent antifungal compound, comparable to the standard Nystatin (Silva et al., 2010).

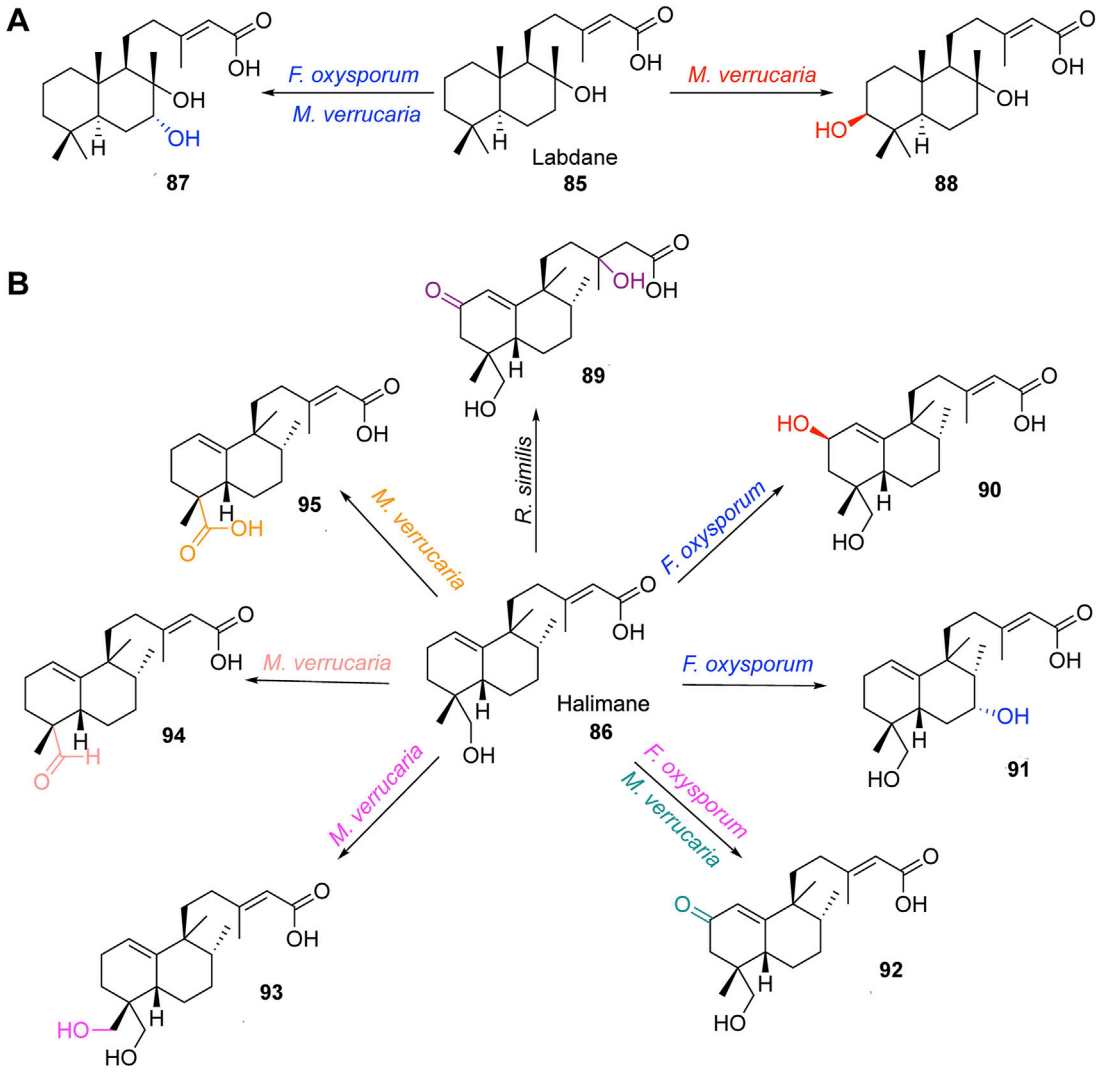


FIGURE 6
Biotransformations explored for labdane **(A)** and halimane **(B)** terpenes.

Phomopsis sp. and *Phaeoacremonium* sp. were two fungi isolated from *S. spectabilis* leaves. The study involving *Phaeoacremonium* sp. led to the discovery of three new isoaigialones (A–C; **75**–**77**), along with the known aigialone (**78**). Compounds **76** and **78** exhibited antifungal activity comparable to the standards Nystatin, and moderate activity against human cervical tumor cell line (HeLa) in the MTT assay (Silva et al., 2017). Among the compounds isolated from *Phomopsis* sp., cytochalasins J (**79**) and H (**80**), in addition to alternariol (**81**) stand out as they showed potent inhibition of reactive oxygen species (ROS) produced by stimulated human neutrophils suggesting their potential as anti-inflammatory agents. Moreover, **80** also demonstrated antifungal activity and AChE inhibition (Chapla et al., 2014).

Traditional investigations of microbial secondary metabolites often focus on identifying bioactive compounds using a single culture medium. However, several biosynthetic gene clusters might remain silent or cryptic under laboratory conditions, limiting the discovery of additional natural products (Pinedo-Rivilla et al., 2022). To overcome this challenge, researchers have adopted the "One Strain, Many Compounds" (OSMAC) (Bode et al., 2002) approach. OSMAC involves cultivating microorganisms under diverse culture conditions, including variations in temperature, pH, salinity, media composition, and aeration. In addition, by introducing chemical elicitors, epigenetic modifiers, or co-cultivation of different species, it is possible to uncover a wider range of potentially bioactive compounds (Pinedo-Rivilla et al., 2022). These strategies have proven to be valuable in exploring the hidden metabolic potential of microorganisms. Recent studies carried out in NuBBE employed the OSMAC approach in combination with the Design of Experiments (DoE) to induce metabolic variability in *Xylaria* sp. (Vieira et al., 2021) or to optimize the production of bioactive compounds by *Fusarium oxysporum*, *Xylaria cubensis*, and *Diaporthe anacardii*. This study showed that the production of three bioactive compounds, namely fusaric acid (**82**), cytochalasin D (**83**), and 3-nitropropionic acid (**84**) could be significantly enhanced under specific conditions. Notably, the production of **84** was increased by up to 33% (Selegato et al., 2019).

Another line of research explored in NuBBE is the biotransformation method, which uses endophytic fungi to carry out chemical modifications in compounds (Zhang et al., 2021). Studies conducted with three different fungi strains demonstrated the feasibility of obtaining labdane (**85**) and halimane (**86**) derivatives (Monteiro et al., 2017; Monteiro et al., 2019) (Figure 6). The incubation of diterpene **85** (0.2 mg/mL) with *Fusarium oxysporum* resulted in the production of 7α-hydroxy-labdane (**87**). Conversely, the incubation with *Myrothecium verrucaria* yielded both **87** and 3β-hydroxy-labdane (**88**) derivatives. The experiment was conducted for 6 days, under agitation (110 rpm) at approximately pH 5.3, in a CZAPEK medium at 28°C. Compound **88** exhibited enhanced selectivity in the inhibition of anticholinesterase activity (Monteiro et al., 2017).

The biotransformation of halimane **86** (0.4 mg/mL) was investigated through incubation with *F. oxysporum, M. verrucaria*, and *Rhinocladiella similis* in CZAPEK medium for 3 days, under agitation (110 rpm) at approximately pH 5.3°C and 28°C. Seven derivatives from diterpene (**86**) were identified, including four previously known compounds (**92**–**95**) (Monteiro et al., 2017) and three new compounds (**89**, **90**, **91**). Compound **86** presented a moderate reduction of a biofilm generated by *Staphylococcus epidermidis* ATCC35984, being this effect also observed in **91** and **95** (Monteiro et al., 2019). These studies led to the conclusion that important structural modifications, such as oxidations of inactive carbon sp^3, yielded analogous compounds with new reactive sites, showcasing the capacity of endophytic fungi to serve as versatile catalysts for a wide variety of reactions.

Endophytic fungi from marine organisms

More than 70% of the Earth's surface consists of oceans, considered one of the most diverse and underexplored ecosystems. They comprise habitats with physical and chemical characteristics very different from land-based ecosystems, reflecting a large chemodiversity and a variety of bioactive secondary metabolites (Cappello and Nieri, 2021; Pinedo-Rivilla et al., 2022). Research on marine natural products began in the 1940s when the D-arabinose nucleosides spongouridine and spongothymidine were isolated from the sponge *Tethya* or *Cryptotethya crypta*. These compounds inspired the synthetic development of cytarabine (Ara-C) and vidarabine (Ara-A), commercialized as Cytosar-U® and Arasena A®, which are drugs approved for the treatment of acute myeloid leukemia and herpes virus infection, respectively (Oliveira et al., 2012; Cappello and Nieri, 2021). To date, there are about fifteen approved marine-derived drugs (Marine Pharmacology, 2023). More recently, in 2021 alone, 1,425 new natural products were described from marine sources, including alkaloids, polyketides, terpenes, peptides, macrolides, steroids, and lactones derivatives, with several bioactivities reported, such as antifungal, antibacterial, antileishmanial, anti-tubercular, cytotoxic, antiviral and anti-inflammatory properties (Carroll et al., 2023).

Between 1977 and 2019, about 5,500 compounds from marine fungi were described, of which around 3,000 have been reported between 2015 and 2019, revealing a remarkable diversity of bioactive secondary metabolites with pharmacological or biotechnological potential (Calado et al., 2021; Carroll et al., 2021; Carroll et al., 2023). Marine endophytic fungi present great chemical and biological potential due to their ecological relationship with the host. The fungal endosymbionts have been found in seagrass, marine invertebrates (such as sponges and corals), and mostly in algae (Oliveira et al., 2012; Teixeira et al., 2019; El-Bondkly et al., 2021). The endosymbionts mycobiota is affected by host species and genotype, tissue origin, geography location, nutrient availability, or other abiotic and biotic stress, resulting in a variety of species associated with one host (El-Bondkly et al., 2021).

The Brazilian sea coast extends for 7,491 km and comprises three marine ecosystems and 12 major hydrographic regions (MMA, 2023), representing a huge potential for the study of natural products of marine origin. The studies of endophytic fungi from marine organisms in NuBBE began in 2009, with the first collections of algae on the north coast of the São Paulo state, on Fortaleza beach (Ubatuba, SP). From this collection, the fungus *Penicillium citrinum* was isolated from the red alga *Dichotomaria marginata*, being possible to isolate the alkaloids quinolactin A (**96**), B1 (**97**), B2 (**98**) and D (**99**) (Andrade et al., 2013), the quinolizidine alkaloids citrinadin A (**100**) and B (**101**), 18-dehydroxy-citrinadin B (**102**), and chrysogenamide (**103**) (de JASAndrade et al., 2014) and the polyketides citrinin (**104**) and dicitrinin-A (**105**) (Figure 7). These last two metabolites showed toxic effects on *Artemia salina* in addition to cytotoxic and mutagenic effects on *Allium cepa*. Moreover, these polyketides modulated H_2O_2-induced oxidative damage in *Saccharomyces cerevisiae* strains (de Oliveira Filho et al., 2022). Citrinin exhibited cytotoxic and antitumor effects on Sarcoma 180 (S-180) cultures, indicated by increased apoptosis and necrosis of S-180 cells (de Oliveira Filho et al., 2020). It also showed a reduction of 7,12-dimethylbenzanthracene (DMBA)-induced mammary carcinoma cell proliferation in Swiss mice, with no behavior and locomotor alterations (de Oliveira Filho et al., 2021).

Further investigation on this topic led to the study of the endophytic fungal strains *Annulohypoxylon stygium*, *Annulohypoxylon yungensis*, and *Nemania bipapillata*, isolated from specimens of the *Falkenbergia* stage of the red alga *Asparagopsis taxiformis*, also collected in 2013, at the rocky coastal area of Fortaleza Beach (Ubatuba, SP, Brazil) (Medina et al., 2019a; Medina et al., 2019b). A bio-guided fractionation of the crude extract obtained from *A. stygium* and *A. yungensis*, with a focus on antimicrobial activity, led to the isolation of the tetralone derivatives (3*R*)-scytalone (**106**), and (3*R*,4*R*)-4-hydroxy-scytalone (**107**) from the second strain and pyrogallol (**108**) from the first strain. Pyrogallol was active against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* strains (Medina et al., 2019a). In addition, (3*R*,4*R*)-3,4,5-trihydroxy-1-tetralone (**109**) and tyrosol (**110**) were isolated from an inactive fraction obtained from *A. stygium* (Medina et al., 2019a). For *N. bipapillata*, a fractionation yielded three new botryane sesquiterpenes (**111**–**113**) and one pair of diastereomeric botryane *nor*sesquiterpenes bearing a new degraded carbon skeleton (**114** and **115**), along with the known 4β-acetoxy-9β,10β,15α-trihydroxyprobotrydial (**116**). The compounds were tested as cholinesterase inhibitors and nemenonediol A [(+)-(2*R*,4*R*,8*R*)-(8)] weakly inhibited acetylcholinesterase, while (+)-(2*R*,4*S*,5*R*,8*S*)-4-deacetyl-5-hydroxy-botryenalol (**111**) inhibited both

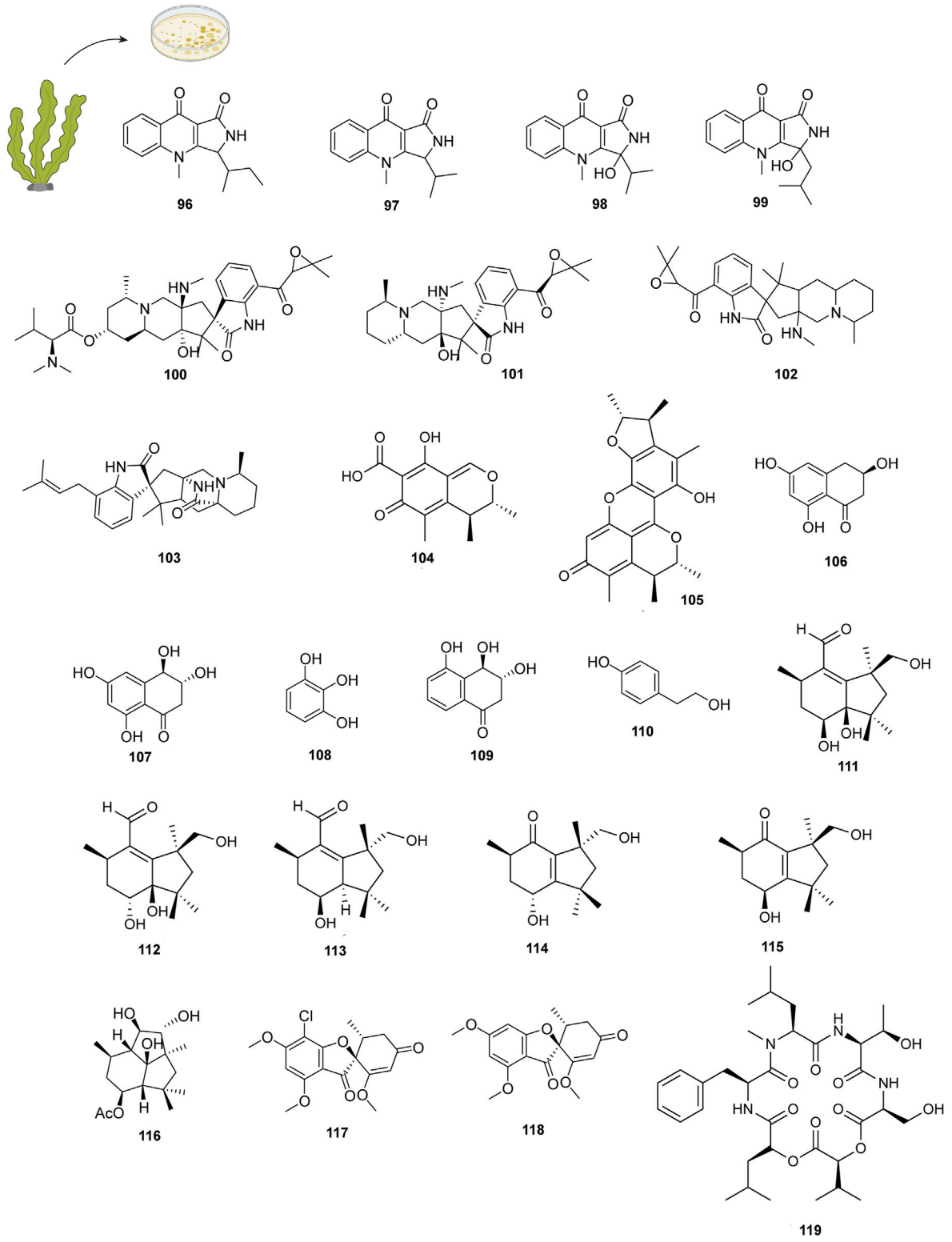


FIGURE 7
Compounds isolated from *Penicillium citrinum* (**96**–**105**), *Penicillium chrysogenum* (**117**–**119**), *Annulohypoxylon yungensis* (**106** and **107**) and *A. stygium* (**108**–**110**), and botryane terpenoids (**111**–**116**) isolated from *Nemania bipapillata*. Icons were obtained from BioRender.com.

acetylcholinesterase and butyrylcholinesterase. Compounds **111**, (+)-(2*R*,4*S*,5*R*,8*R*)-4-deacetyl-botryenalol (**113**), nemenonediol B [(+)-(2*R*,4*S*,8*S*)-(9)], and 4β-acetoxy-9β,10β,15α-trihydroxyprobotrydial (**116**) were tested against colorectal carcinoma HCT-116 and breast adenocarcinoma MCF-7 cell lines, but presented no significant toxicity at the tested concentrations. The new compounds had their absolute

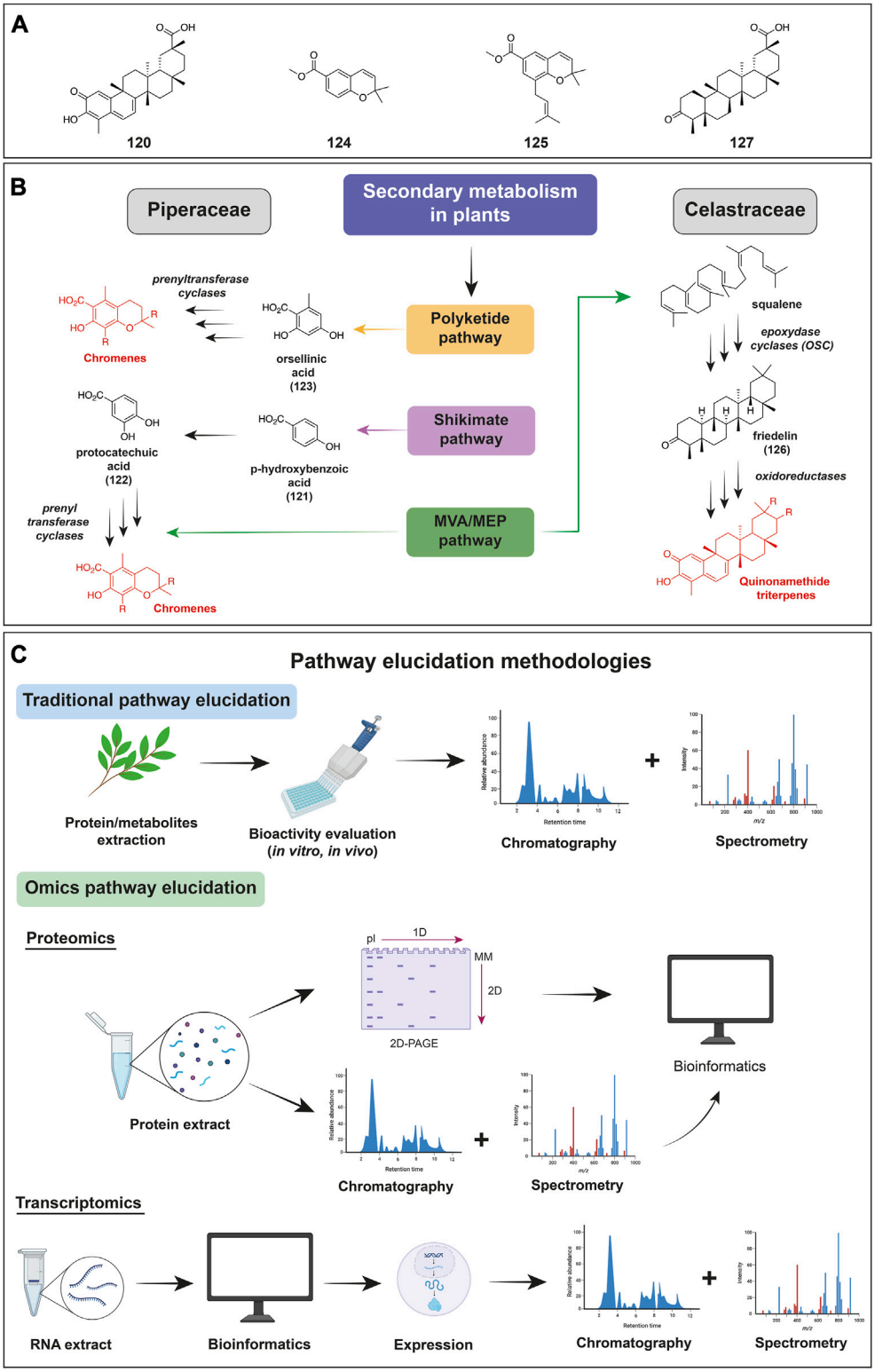


FIGURE 8
Quinonemethide triterpenes (**120**, **127**) from Celastraceae species and chromenes (**124**, **125**) from *Piper* species **(A)**. Summary of biosynthetic studies explored by NuBBE over the past 25 years **(B)**. Piperaceae and Celastraceae biosynthetic pathways were investigated through **(C)** traditional phytochemical studies and also by omics approaches (proteomics and transcriptomics). Icons were obtained from BioRender.com.

configurations determined by comparison of experimental and calculated electronic or vibrational circular dichroism data (Medina et al., 2019b).

Another study conducted in the group with focus on *Penicillium chrysogenum*, a fungus from the brown alga *Padina gymnospora*, resulted in the isolation of the polyketides griseofulvin (**117**) and 7-dechlorogriseofulvin (**118**), in addition to the cyclohexadepsipeptide hirsutatin A (**119**). Hirsutatin A exhibited anti-inflammatory activity, indicated by the dose-dependent inhibition of NO overproduction (de JASAndrade et al., 2015).

Biosynthetic studies

In addition to the isolation and bioactivity evaluation of natural products, the NuBBE research group has also been committed to contributing with in-depth knowledge of the biosynthesis of secondary metabolites of different plant families, particularly Piperaceae and Celastraceae species (Figure 8). Although Piperaceae is known for accumulating different classes of secondary metabolites, the prenylated benzopyrans (chromanes and chromenes) stand out for being responsible for a significant number of reports of biological activities (López et al., 2010). Concerning Celastraceae species, special attention worldwide is growing on the quinone methide triterpenes, such as celastrol (**120**) and pristimerin (**17**), mainly due to their anticancer, anti-inflammatory, anti-conceptive, and antimicrobial properties (Bicalho et al., 2019).

The biosynthesis of prenylated benzopyrans compounds follows two distinct routes, one derived from the shikimic acid pathway and the other derived from the polyketide pathway (Kato and Furlan, 2007). For *Piper* species, the chromenes come from the shikimate pathway, which uses *p*-hydroxybenzoic (**121**) or protocatechuic acid (**122**) as precursors. The next step in the biosynthesis of these metabolites involves the prenylation of the phenylpropanoid unit (Lopes et al., 2007). However, for *Peperomia* species, the chromanes are biosynthesized by orselinic acid (**123**), a polyketide commonly found in endophytic fungi (Kato and Furlan, 2007).

Several studies were carried out in order to contribute to the elucidation of the steps involved in the biosynthesis of these compounds. In 2005, the enzymatic conversion of methyl 2,2-dimethyl-2H-1-chromene-6-carboxylate (**124**) into methyl 2,2-dimethyl-8-(3′-methyl-2′-butenyl)-2H-1-chromene-6-carboxylate (**125**) in *Piper aduncum* leaves was investigated. This work concluded that the prenyltransferase activity, which catalyzes the transfer of the dimethylallyl group to the C-2′ of **123**, depends on circadian variation (Morandim et al., 2005). López and coworkers evaluated the activity of geranyltransferases in aromatic substrates using enzymatic extracts of *Piper crassinervium* leaves (López et al., 2010).

The biosynthetic origin of the isoprenoid units of the chromenes of *P. aduncum* and *Piper gaudichaudianum* was also investigated to determine which pathway, the mevalonate (MVA) or the pyruvate-triose (MEP) non-mevalonate, was active in the biosynthesis of these compounds. For this, metabolic studies involving the incorporation of [1–^{13}C]-D-glucose into leaves of these species were performed (Leite et al., 2007; Lopes et al., 2007). The results have indicated that both the MVA and the MEP pathways are implicated in the biosynthesis of isoprene moieties present in these compounds.

The biosynthesis of chromanes and chromenes also involves a cyclization step that gives rise to the benzopyran ring. Two studies involving proteomic and/or transcriptomic analyses were performed with *P. gaudichaudianum* and *Peperomia obtusifolia* aiming to describe the main biosynthetic pathways active in these species, in addition to identifying key enzymes involved in these processes (Batista et al., 2017; Batista et al., 2018). Both studies have revealed the presence of prenyltransferases and tocopherol cyclase enzymes, which may be responsible for the prenylation and cyclization that yield the prenylated benzopyrans. In addition, the transcriptomic study of *P. gaudichaudianum* allowed proposing that the precursor *p*-hydroxybenzoic acid (**121**) is biosynthesized via the β-oxidative pathway in this *Piper* species (Batista et al., 2018). Finally, the fact of not identifying polyketide synthases in *P. obtusifolia* suggests that the orsellinic acid (**123**) may be produced by endophytes associated with the plant (Batista et al., 2017).

Different species of the Celastraceae family also aroused interest in understanding the chemistry and the routes involved in the biosynthesis of their secondary metabolites. For instance, the native Brazilian medicinal plant *Maytenus ilicifolia* Mart. ex Reissek (popularly known as espinheira santa) is widely used in traditional folk medicine to treat gastric ulcers (Bicalho et al., 2019) and therefore has been investigated under chemical and biosynthetic aspects. Among the secondary metabolites accumulated in *M. ilicifolia*, the quinone methide triterpenes (QMT) were studied from the biosynthetic point of view. This class of metabolites is responsible for important bioactivities reported for the species (Costa et al., 2008; Dos Santos et al., 2010; Santos et al., 2012; Jardim et al., 2015; Bicalho et al., 2019). QMT is biosynthesized in the root barks of the species and accumulates during youth, yet in small amounts, and its biosynthesis is associated with the production of the pentacyclic triterpene friedelin (**126**) in leaves (Corsino et al., 2000; Buffa Filho et al., 2002). Friedelin (**126**) is biosynthesized from 2,3-oxidosqualene, which is derived from isopentenyl pyrophosphate generated through the mevalonate pathway (Corsino et al., 2000; Souza-Moreira et al., 2018; Bicalho et al., 2019). The cyclization of 2,3-oxidosqualene to friedelin (**126**) is catalyzed by a specific oxidosqualene cyclase (OSC), the friedelin synthase, which is responsible for the highest number of rearrangements in the triterpene biosynthetic pathways (Alves et al., 2018; Mazzeu et al., 2021). QMT pathway is not simple, and many other enzymes are involved in it. The proposition of the pathway has celastrol (**120**) as the first QMT, but its presence is not noteworthy in *M. ilicifolia*. This suggests its further oxidation to pristimerin (**17**) and maytenin (**16**), other very important chemicals with different biological activities, including anticancer (Cevatemre et al., 2018).

An overview of the enzymes involved in this route guided us to the amplification of the coding sequence of friedelin synthase from the leaves of *M. ilicifolia* (MiFRS, Genbank accession number KX147270). Further, its function was characterized in the yeast *Saccharomyces cerevisiae*, a microorganism with the advantages of a eukaryotic cell that synthesizes the substrate oxidosqualene (Souza-Moreira et al., 2016). Differences in the friedelin synthase full-length coding sequences amplified were noticed, which guided distinguishing three isoforms of the enzyme in *M. ilicifolia* with similar production in yeast (Alves et al., 2018).

Moreover, the alignment of OSC and *in silico* analysis are efforts to understand the oxidosqualene cyclization to a ketonic structure and to search for a mutant with higher productivity of friedelin (**126**). These analyses revealed residues that are essential to the oxidosqualene cyclization activity, in addition to others involved in the specificity of the enzyme's product (Souza-Moreira et al., 2016; Mazzeu et al., 2021). Interestingly, some of these residues can contribute for the enzyme to stabilize the intermediates, resulting in a higher friedelin production compared to the wild type (Mazzeu et al., 2021). This suggests the possibility of exploring friedelin synthase engineering to find a higher producer. As precursor of QMTs, its improved production in yeast could facilitate the elucidation of the QMT pathway. Additionally, yeast metabolic engineering could make *S. cerevisiae* a future cell producer of friedelin due to its biological activities.

Continuing the research on the QMT biosynthetic pathway of *M. ilicifolia*, transcriptomic analysis of the roots led to the annotation of a CYP712K4 oxidoreductase (Genbank accession number MK829814). Its functional expression in *Nicotiana benthamiana* leaves and *S. cerevisiae* has proven the oxidation of friedelin (**126**) at carbon 29, resulting in maytenoic acid (**127**) as the main product, through a three-step oxidation process involvingalcohol and aldehyde groups. CYP712K4 is therefore claimed as the first oxidation step in the QMT biosynthesis that is about to be elucidated (Bicalho et al., 2019).

It is important to note that in QMT pathway, many other enzymes are involved until pristimerin (**17**) and maytenin (**16**) in *M. ilicifolia*. The NuBBE research group is committed to deciphering the pathway of QMT biosynthesis and has provided valuable information on the precursor friedelin (**126**) and the first oxidation step characterized by the enzymes friedelin synthase MiFRS and CYP712K4. The continuing efforts of the group are focused on the elucidation of the QMT pathway, allowing for the sustainable production of QMTs in robust organisms such as *S. cerevisiae*.

Medicinal chemistry

The NuBBE research group has also been actively involved in the field of synthetic and medicinal chemistry. More specifically, the research focuses on compounds inspired by natural products, in addition to semi-synthetic ones, aiming to improve their bioactivities with a particular emphasis on inflammatory diseases, neglected diseases, and with anticancer properties.

During the Research Program in Characterization, Conservation, Restoration and Sustainable Use of Biodiversity (*Pesquisas em Caracterização, Conservação, Restauração e Uso Sustentável da Biodiversidade*–BIOTA) thematic program, a throughput screening was performed aiming to find novel compounds as anti-inflammatory alternatives (Fernandes et al., 2008; Zeraik et al., 2012). A series of *O*-glycosyl-flavones (**128**–**133**) isolated from *Pterogyne nitens* presented outstanding inhibition of myeloperoxidase (MPO) in the nanomolar range. Although the *O*-glu substituent increases the MPO inhibition (IC_{50} = 15.8 nM) compared to *O*-glu-*O*-rha (IC_{50} = 25 nM), the presence of both at position C-6 is well tolerated for biological activity. In addition, the hydroxyl group at position C-3 improved MPO inhibition when comparing compound **131** (IC_{50} = 22 nM) with flavone **132** without the *O*-glucosyl group (IC_{50} = 3.8 nM) (Fernandes et al., 2008) (Figure 9A).

These results led to a deeper investigation of flavonoids as key scaffolds for MPO inhibition. Therefore, chalcones (open-chain flavonoids) were synthesized (**134**–**136**) by varying electron donating and withdrawing groups to establish a structure-activity relationship (SAR) following these bioisosteric and molecular simplification strategies: 1) changing hydroxyl group at C-6 to an amine at para-position; 2) ring C opening; and 3) variation of groups at ring A. Just one amine group at para-position was enough to reach compounds with nanomolar potency at MPO inhibition, once methyl and fluorine substituents at para-position of ring A did not enhance the activity (Zeraik et al., 2012) (Figure 9B).

The discovery of new compounds with antiparasitic activities are also of great interest, with a particular focus on leishmaniasis, Chagas disease, and malaria–all neglected diseases that affect several developing countries, including Brazil. Among the compounds described by NuBBE, prenylchalcones exhibited notable antileishmanial activity. A series of *O*-prenylchalcones (**137**–**140**) was synthesized to investigate the effects of both position and carbon numbers of prenyl group over antileishmanial activity (Figure 9C). *O*-prenylation at position 2 and a farnesyl group (15 carbon atoms) were found to be pivotal for this biological activity compared to prenyl and geranyl groups (5 and 10 carbon atoms, respectively). Chalcone **138** was up to 8-fold more potent than pentamidine against amastigote forms from *Leishmania amanozensis* (2.90 μM *versus* 6.25 μM) and *Leishmania infantum* (2.24 μM *versus* 19.77 μM) (Passalacqua et al., 2015a). It was suggested that higher lipophilicity of compound **138** is responsible to that potency, since its LogP calculated was founded to 7.75 compared to 4.69 for compound **137.**

Some hydroxy-chalcones also play a role in the reduction of leishmania forms, mainly in the inhibition of pivotal targets. During our screening programs of compounds against leishmania, 2′,4′-dihydroxychalcone (**141**) demonstrated IC_{50} value of 0.4 μM against promastigotes (Figure 9D). Through virtual screening against leishmania targets, this compound revealed interactions with glycerol-3-phosphate dehydrogenase (G3PDH) (Passalacqua et al., 2015b). The binding mode shows mainly polar interactions as flagship between G3PDH and 2,4-dihydroxychalcone, since carbonyl group and both hydroxyl interacts with Arg 274 and Val 92, Glu 300 and Lys 125 respectively. Particularly, the computational chemistry study was pivotal to determine the binding mode between biological target and chalcone, which could boost further investigations to find more potent compounds against *Leishmania*.

Several amides and chromene compounds with potent trypanocidal activity were isolated from *Piper gaudichaudianum* and *P. aduncum*. Dihydropyridone amides were isolated as isomers (*E*, *Z*), to which (*Z*)-piplartine (**142**) was 2-fold potent (IC_{50} = 10.5 μM) compared to (*E*)-piplartine (**143**, IC_{50} = 21 μM) and clinical drug benznidazole (IC_{50} = 42 μM). Moreover, the hydrogenation (**144**) of vinyl group in (*E*)-piplartine reduced the trypanocidal activity against epimastigote forms by 3-fold (Cotinguiba et al., 2009) (Figure 9E). In addition, di-prenylated chromenes also has been identified as promising trypanocidal agents. For these compounds, the presence of ester group is 10-

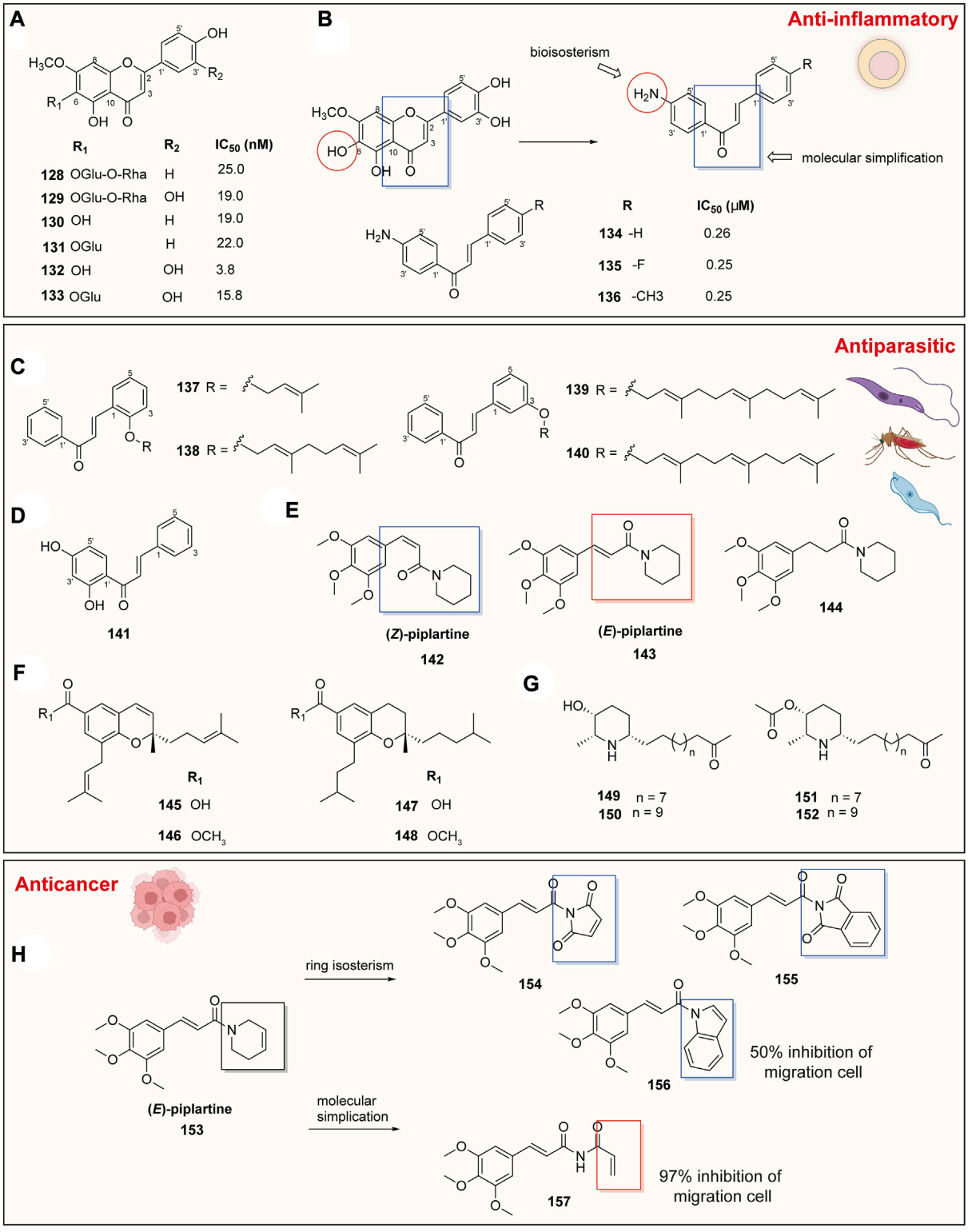


FIGURE 9
Compounds investigated on medicinal chemistry projects in the NuBBE research group. **(A)** Glucosil and non-glucosil-flavones isolated from *Pterogyne nitens* as MPO inhibitors. **(B)** Biososterism and molecular simplification strategies applied to design synthetic aminochalcones as MPO inhibitors. **(C)** Synthetic prenylchalcones founded with antileishmanial and antichagasic activity. **(D)** Chemical structure of 2′,4′-dyhydroxychalcone with antileishmanial activity. **(E)** Amides isolated from *Piper gaudichaudianum* with trypanocidal activity. Amide piplartine was described as (*E*,*Z*) isomers, to which **(E)**-isomer is more promising besides hydrogenation to reduce the activity. **(F)** Prenylchromenes islated from *P. aduncum* with trypanocidal activity. Chromenes containing an ester group are more potent than compounds with an acid group. **(G)** Piperidine alkaloids from *Senna spectabilis* activity against *Plasmodium falciparum*. Compound (−)-spectaline without acetyl group has outstanding low micromolar range of potency against the parasite. **(H)** (*E*)-piplartine derivatives designed and synthesized as inhibitors of metastatic cancer. Compounds containing acrylamide group had outstanding inhibition of cell migration (MDA-MB-231) (Valli et al., 2017). Icons were obtained from BioRender.com.

fold more potent than chromenes containing a carboxylic acid against epimastigote forms (compounds **145** and **146**). The trypanocidal effectiveness is lost when the double bond in both the prenyl group and heterocycle ring is hydrogenated (Compounds **147** and **148**), making vinyl essential to that effect (Batista et al., 2008) (Figure 9F).

Regarding antimalarial activity, the piperidine alkaloids (−)-cassine (**149**) and (−)-spectaline (**150**), isolated from *Senna spectabilis* (D.C), were identified as promising compounds, with potent inhibition of *Plasmodium falciparum* (IC_{50} of 1.84 μM and 2.76 μM, respectively). However, *O*-acetylation of these compounds (**151** and **152**) reduced their potency by up to 13 times compared to chloroquine (0.30 μM) (Pivatto et al., 2014) (Figure 9G).

The global pursuit of novel strategies to address the challenges posed by cancer metastasis remains a significant concern, and compounds from natural sources are a great inspiration for this quest. (*E*)-piplartine (**153**) is a promising natural compound with anticancer activity identified in NuBBE, isolated from *P. gaudichaudianum* (Cotinguiba et al., 2009). Through biosisterism and molecular simplification of the heterocycle ring (Figure 9H), the electronic properties of (*E*)-piplartine (**153**) were explored in a metastatic cell model assay. Among the derivative compounds, compound **156**, which contained phthalimide and indole rings generated by ring isosterism, exhibited 50% inhibition of cell migration at 10 μM in a breast adenocarcinoma model (MDA-MB-231) (Valli et al., 2017). Additionally, molecular simplification resulted in compound **157**, containing an acrylamide moiety, which demonstrated a remarkable inhibition of 97% of cell migration for MDA-MB-231 cells. Moreover, this modification also resulted in slightly reduced cytotoxicity compared to (*E*)-piplartine (**153**) (Valli et al., 2017).

The NuBBE research group has made significant contributions to medicinal chemistry by uncovering new bioactive compounds, including semisynthetic and natural product-inspired molecules, providing valuable scaffolds for drug design. The availability of these compounds in the NuBBE database ($NuBBE_{DB}$) serves as a valuable resource for both academic institutions and companies. Researchers can leverage this database to design and develop promising drug candidates across various areas including the ones mentioned above.

Development of NuBBE database

Brazilian researchers have been studying natural products for over 70 years, and until 2015, the resulting scientific information was dispersed in around 32,000 scientific articles in the literature. Information o these articles was requested on demand and directly received by the database of the National Council for Scientific and Technological Development (CNPq), an organization of the Brazilian Ministry of Science and Technology. To overcome this issue, NuBBE created a dedicated and freely accessible online platform called $NuBBE_{DB}$ (https://nubbe.iq.unesp.br/portal/nubbedb.html) (Valli et al., 2013). This ongoing project aims to create the most comprehensive database of natural products from Brazilian biodiversity, while employing advanced tools to predict and identify bioactive compounds for drug design, chemical ecology, annotation of natural products and cataloging of the chemical diversity across the vast Brazilian territory.

Prospecting this molecular source is one of the most challenging stages of basic research, which connects scientific knowledge and translational research with drug development and applied research. The creation of a Brazilian biodiversity database represents a high-added value product and can be of great use to scientists working in interdisciplinary fields, including chemical, biological, physical, medical, and pharmaceutical sciences, among others (Valli et al., 2018).

The curation of data on the natural products from Brazilian biodiversity began in 2010 and has had a great impact since its launch in 2012 (Figure 10). $NuBBE_{DB}$ is the first collection of natural products from Brazilian biodiversity and is globally recognized (Fapesp, 2013; Medina-Franco, 2013; Villoutreix et al., 2013; Harvey et al., 2015; Ntie-Kang et al., 2017). $NuBBE_{DB}$ gathers information on biological descriptors, chemical properties, molecular structure data, taxonomy, and geographic location data, making it unique compared to other natural product databases available worldwide (Valli et al., 2013). The ongoing $NuBBE_{DB}$ project is important for several strategic areas of biodiversity mapping, allowing access to and sustainable use of biodiversity in the search for bioactive compounds. Databases are essential for integrating experimental and computational techniques for biological assays, large-scale screenings (experimental and virtual), and construction of property prediction models.

$NuBBE_{DB}$ is currently undergoing an expansion through its second and third stage projects. A list containing 32,524 scientific articles published between 1950 and 2015 by researchers in the area of natural products and cataloged on the Lattes platform (http://lattes.cnpq.br/) was provided by CNPq as part of this project. This list was used to filter articles with potential data on natural products from Brazilian biodiversity. In 2017, a new version of the database was launched, with more than 2,000 compounds (Pilon et al., 2017).

The project is currently in association with the Chemical Abstracts Service (CAS), a division of the American Chemical Society (ACS), renowned for its expertise in cataloging chemical information [CAS, 2020]. This collaboration aims to accelerate data compilation to expand $NuBBE_{DB}$ content. While features to automate part of the data compilation processes are very useful, the verification of data by expert scientists is essential to ensure the reliability and quality of the data. The data received by CAS constitutes part of the data, and the researchers involved in this project also access the original articles to extract additional information, such as the species from which the compounds were isolated, geographic location of the species collected, metabolic class, and biological activity. These details are not compiled by CAS, making individual verification of the original articles necessary.

Maintaining a database is a complex and long-term project, and currently, about 40% of the information referring to studies of natural products from Brazilian biodiversity has been compiled. Even though the compilation of this database is a significant achievement, its impact goes far beyond only cataloging information. The extraordinary chemical space covered by Brazilian natural products stands out as an inspiring source of innovative molecules for the discovery of new drugs and products of pharmaceutical interest. The structural diversity and the wideness of the chemical space of $NuBBE_{DB}$ show an adequate

FIGURE 10
NuBBE Database development, highlighting the milestones from 2012 to 2018. Icons in the figure were obtained from flaticon.com.

pharmacodynamic and pharmacokinetic profile for the discovery of drug candidates with high potential for clinical development. This means that the database contains structures with molecular and physicochemical characteristics similar to those of approved drugs. This finding highlights that the natural products database is an inspiring source of molecules for drug discovery and studies in medicinal chemistry (Saldívar-González et al., 2019), underscoring the reach and merit of national science on the global stage.

The most recent project involving $NuBBE_{DB}$ aims to improve data sharing by implementing taxonomies and ontologies, making it easier for people and computers to access and use the information. Following the FAIR principle of making data (Findable, Accessible,

Interoperable, and Reusable) (Wilkinson et al., 2016), the project will focus on developing a standardized language for the $NuBBE_{DB}$ domain by investigating the existing taxonomies used by ChEMBL and PubChem. Another objective of this project is to develop target prediction tools, which will facilitate drug discovery research.

Further than its high impact on national science, the database of natural products from Brazilian biodiversity, $NuBBE_{DB}$, has attracted significant international interest during the 10 years of its existence due to its originality, high quality, creativity, and innovation. The innovation of $NuBBE_{DB}$ contributes to national socioeconomic development by promoting the intelligent and sustainable use of Brazilian biodiversity, generating a positive impact in the society.

Tools and methodologies for phytochemical and data analyses

The chemical study of natural products is far from being a simple task. The high complexity of the sample matrix, the wide range of polarities and concentrations of primary and secondary metabolites, and the challenging compound annotation and identification require the use of very well-defined protocols from the early steps of sample acquisition, passing through sample preparation and data acquisition, until data analysis (Bueno and Lopes, 2020).

However, over the past 25 years, the research of natural products was greatly benefited thanks to the development of new and improved sample preparation protocols, including the increasing availability of high-resolution separation and state-of-the-art analytical techniques and instrumentation, combinations like LC-MS, CE-MS, LC-NMR, or LC-DAD-SPE-NMR-MS, as well as novel algorithms, cheminformatics, and bioinformatics tools and workflows. These techniques made it possible for the annotation, the identification, and even the full structural elucidation of both known or new lead compounds, without the employment of time-consuming isolation and purification protocols in a significantly reduced time and using considerably lower sample amounts (Silva et al., 2022).

Undoubtedly, the proper credit must be given to the classic phytochemistry methodologies, which allowed us to build the base of the modern metabolomics approaches, which have been even more used nowadays. By using traditional extracts preparation, fractionation, and compound isolation, hundreds of compounds had their structure fully elucidated and fed the modern and curated compounds databases, boosting the development of new search algorithms and computational tools for extract dereplication and fast compound annotation (Pilon et al., 2020; Silva et al., 2022).

Although being one of the grounds supporting the natural products chemistry, both traditional phytochemical methods and the recent modern metabolomics approaches have drawn the community's attention to the use of environmentally friendly solvents and green protocols for sample preparation and analysis. With this regard, NuBBE has been investing many efforts in the use and development of eco-friendly protocols, avoiding the use of organic and halogenated solvents for sample extraction and fractionation, along with the use of DoE as rational strategies to minimize the consumption, disposal and the negative impact of hazardous chemicals (Funari et al., 2019; Vieira et al., 2021; Souza et al., 2022). An interesting example is the development of a green ultra-high performance liquid chromatography method using sustainable sugar cane grade ethanol as a solvent for the fingerprint analysis and discrimination of a set of propolis samples from seven countries, assisted by principal component analysis and partial least squares–discriminant analysis (Funari et al., 2016).

Following the greener approaches, research about the use of natural deep eutectic solvents (NADES) can be highlighted. NADES are mixtures of natural compounds such as sugars, organic acids, amino acids, and organic bases that are abundant in organisms (Funari et al., 2019) that can be used as an alternative to organic solvents for the extraction of natural compounds of different polarities, while being inexpensive, non-toxic, and easy to prepare. Although their conception is similar to conventional synthetic ionic liquids (ILs) and deep eutectic solvents (DESs), NADESs are cheaper and have acceptable toxicity and lower environmental impact, in addition to negligible volatility and a wide polarity range (Fraige et al., 2019).

Besides its use in sample extraction protocols, eco-friendly solvents such as ethanol, acetone, and NADES can also be used as greener replacements for the use of organic modifiers and solvents in reversed-phase high-performance liquid chromatography (RP-HPLC). As an alternative to the use of acetonitrile (ACN) and methanol (MeOH), which have been the most employed organic solvents in RP-HPLC, NADES demonstrated similar chromatographic performances in between those observed for ACN and MeOH, mainly related to eluotropic strength, resolution, and peak capacity (Funari et al., 2014; Funari et al., 2015; Sutton et al., 2018).

Still in the field of greener sample preparation and analysis, NuBBE has initiated the development of an optimized analytical setup in which the extraction of a solid sample is directly coupled to its analysis by high-performance liquid chromatography called Online Extraction Coupled to Liquid Chromatography Analysis (OLE-LC). OLE-LC leads to the elimination of sample preparation steps such as drying, grinding, concentration, dilution, and filtration, among others, and can be easily applied in chromatographic fingerprints and untargeted metabolomic investigations of solid matrices (Ferreira et al., 2016).

Finally, the use of rational DoE, besides contributing to greener analytical methodologies, has been also used to accelerate time-consuming method development for data acquisition in hyphenated techniques involving chromatography and detectors such as UV-DAD or mass spectrometry, to maximize the number of detected peaks (Bueno et al., 2015; Funari et al., 2015). Similarly, the use of instrumental automatization applying the Quality by Design approach and statistical analysis is a valuable approach to investigate complex matrices using a reduced number of runs and time needed. As recently demonstrated by Mannochio-Russo et al. (2020), the entire data collection and method development procedure for the analysis of a mixture of nine Malpighiaceae species extracts comprising screening, optimization, and robustness simulation was accomplished in only 4 days, resulting in very low limits of detection and quantification. The method allowed the successful annotation of 61 compounds, including *O*-glycosylated flavonoids, *C*-glycosylated flavonoids, quinic/

shikimic acid derivatives, sterols, and other phenols, which were efficiently separated by the method developed (Mannochio-Russo et al., 2020).

With the same purpose, which is to increase the Universe of detected peaks and corresponding compounds in analytical separations, comprehensive two-dimensional liquid chromatography (LCxLC) coupled to different detectors such as photodiode array detector (UV-DAD), mass spectrometry (MS) or charged aerosol detector (CAD) has been object of study of the method development team. In the study of Leme and collaborators (Leme et al., 2014), 38 polyphenolic compounds were detected in sugarcane (*Saccharum* spp.) leaf extracts, by using a micro cyano column and a partially porous octadecylsilane column in the first and the second dimension, respectively.

Investments in the use and development of dereplication tools also speeded up the annotation and identification of known compounds in complex raw crude extracts and fractions, accelerating the study of new and unknown compounds as targets in ecological and biological studies of plants and microorganisms. NuBBE also stands out in the use of data obtained from cutting-edge technologies and hyphenated protocols such as liquid chromatography coupled to a diode array detector hyphenated with high-resolution mass spectrometry (LC-DAD-HRMS), or hyphenated with nuclear magnetic resonance (LC-SPE-NMR), or even with tandem mass spectrometry (LC-DAD-MS/MS), as well as with 2D ^{13}C NMR. High throughput screening (HTS), bioinformatics, MS/MS molecular networking applied to the omics sciences, chemometrics, and other statistical tools have been the core of the use of the traditional dereplication methods for the study of plant and microorganisms, and in biological screening processes followed by database searches (Pilon et al., 2020; Pilon et al., 2021).

The accurate annotation of flavonoids using untargeted mass spectrometry analysis, for example, can be done by differentiating similar chemical scaffolds through spectral matching to reference library spectra. In the study of Pilon et al., (2019), combined molecular network analysis, collision-induced dissociation (CID) fragmentation reactions, and chemotaxonomy enhanced the annotation of similar flavonoid glycoconjugates by defining the aglycone nature, the saccharide type, and the number and presence of methoxy substituents of putatively assigned 64 isomers and isobars in the Chrysobalanaceae plant species, most of which are not accurately annotated by automated untargeted MS2 matching (Pilon et al., 2019). Moreover, some of these innovative approaches have demonstrated their critical significance in a chemotaxonomic study conducted on a vast collection of over 100 Malpighiaceae species. In this study, it was possible to establish correlations between various classes of metabolites and distinct phylogenetic clades within this family, shedding light on the intricate relationship between metabolite profiles and the evolution of the species (Mannochio-Russo et al., 2022).

Computational tools

The use of such modern analytical tools, as well as the acquisition of multidimensional data, brought not only essential information for the study of natural products, but also exposed the necessity to develop and use powerful computational tools, compounds databases, algorithms, and pipelines to predict, reveal and integrate the results. An example can be given by the development of a new computational method that analyses ^{1}H NMR data from *F. solani* and *F. oxysporum* isolated from *Senna spectabilis*'s rhizosphere through principal component analysis (PCA), allowing compound dereplication, even in highly similar profiles. As a result, the method, associated with other NMR experiments and information from an in-house *Fusarium*'s metabolite library, was able to distinguish different mycotoxins produced by both fungi, identifying fusaric acid and beauvericin for *F. oxysporum* and the depsipeptide HA23 from *Fusarium solani* (Selegato et al., 2016).

Compounds databases and repositories containing chemical information, such as those acquired from NMR or MS techniques, are indispensable for chemical predictions, dereplication, and structure elucidation. Especially considering the enormous amount of data that can be acquired, the use of *in silico* tools is indispensable to resolve the task. Approaches such as computer-assisted structure elucidation (CASE), pattern recognition methods, and molecular networking (MN) are greatly employed computational methods, assisting compound's identity prediction and structure elucidation of new natural compounds, even in complex mixtures or even when the compound is not present in the database. Together with multivariate data analysis algorithms, these tools proved to be efficient for predicting the bioactivity of compounds by merging the bioactivity of known molecules or fractions (Freire and Castro-Gamboa, 2015; Valli et al., 2019a; Valli et al., 2019b).

In recent efforts conducted in NuBBE, the focus has expanded to include the development of Machine Learning (ML) and Deep Learning (DL) models, facilitated by the expansion of chemical databases, such as NuBBE$_{DB}$. These models use robust computational approaches, such as docking and molecular dynamics, enabled by high-performance computers. *In silico* screening is a cost-effective and high-throughput process that enables computational simulations and robust calculations of chemical structures (Modi et al., 2012), providing flexible tools for analyzing the diversity, biological activity, and toxicity of natural products (Pereira and Aires-de-Sousa, 2018). Combining cheminformatics, ML, and computational power with *in silico* screening can enhance drug discovery by exploring uncharted regions of the therapeutic and chemical space.

ML is a continually evolving field in computer-aided molecule discovery (Lo et al., 2018). Unlike physical models that rely on explicit physical equations or molecular dynamics simulations, ML approaches employ pattern recognition algorithms to identify mathematical relationships between empirical observations of small molecules and predict their chemical, biological, and physical properties (Jeon et al., 2021). For instance, some studies have trained ML models using mathematical mining of chemical entities to generate chemical descriptors for predicting the properties (Medina-Franco and Maggiora, 2013; Fernández-de Gortari et al., 2017). These models recognize structural patterns using molecular descriptors extracted from databases and can even scan molecular images using Convolutional Neural Networks (CNNs) to select relevant features. This approach accelerates insights during computational studies, reducing the cost and time

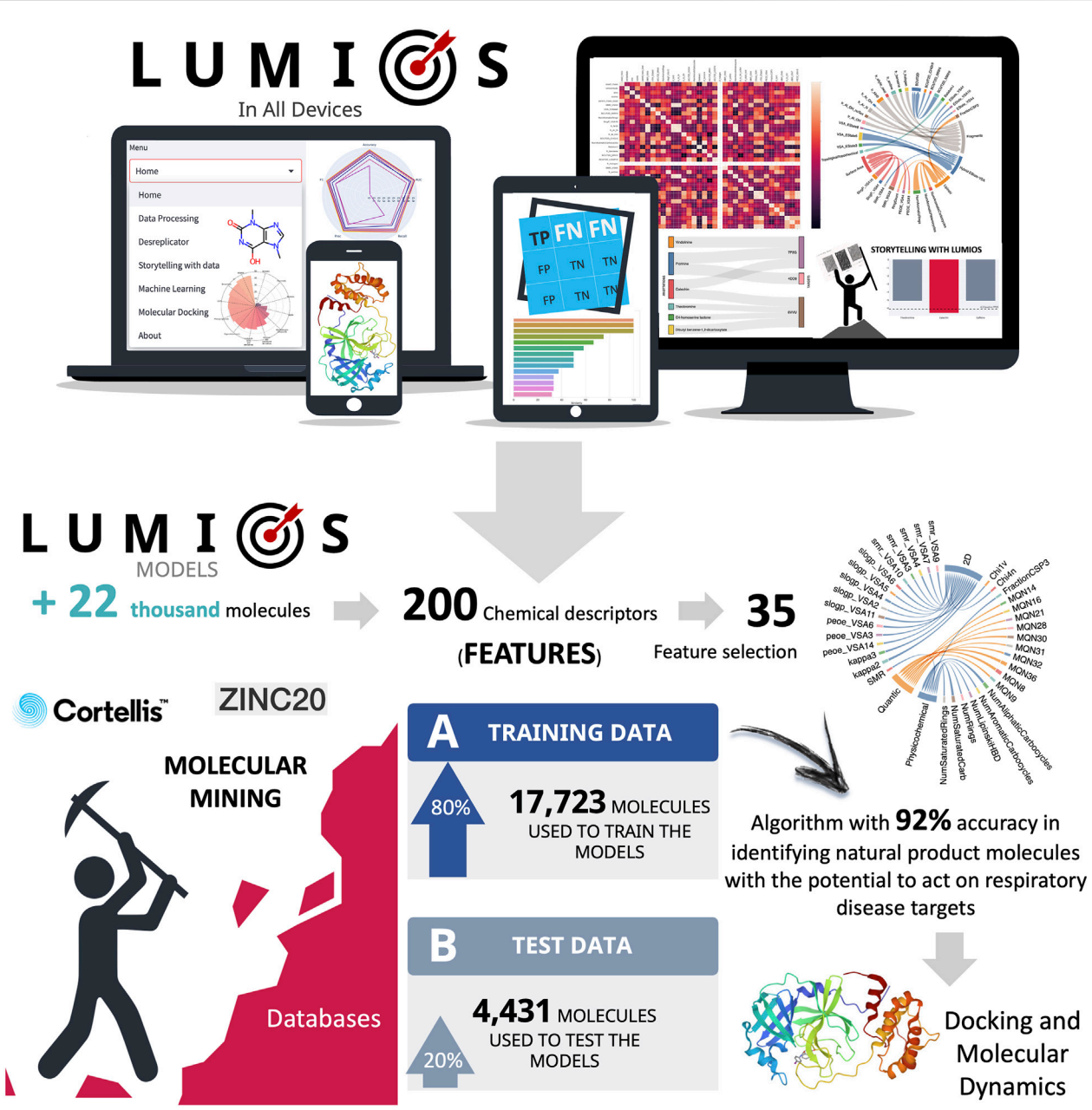


FIGURE 11
Schematic representation of LUMIOS, a multitasking platform developed by researchers at NuBBE. Icons in the figure were obtained from flaticon.com.

involved, and ultimately enhancing the efficiency of exploring natural products.

A software developed in recent years is the LUMIOS platform (Label Using Machine In Organic Samples) (Vieira et al., 2023a). LUMIOS (Figure 11) is a freely accessible tool for molecular analysis, harnessing molecular information obtained through mass spectra preprocessing to facilitate molecular dereplication by comparing spectra with an extensive database of over 1,200,000 chemical compounds. The annotated molecules undergo a rigorous verification filter to ensure accurate classification as natural products. The annotated structures can undergo further analyses using advanced ML and DL models specifically trained to classify molecules as natural products and identify unique patterns exhibited by drug molecules. This integration enables a swift and automated screening process, which proved to be useful for potential biomacromolecular targets associated with respiratory diseases, such as asthma and SARS-CoV-2 (Vieira et al., 2023a).

Perspectives

The natural products field has undergone significant transformations in the past 25 years, propelled by constant changes and scientific advancements. The future of analyzing

complex natural matrices holds great promise and presents opportunities for further progress through the integration of conventional methods and cutting-edge computational tools and technologies (Hu and Qiu, 2023). The use of supercomputers is able to offer massive data processing and analysis capabilities, allowing a broader and more efficient approach to the search for molecules with therapeutic potential, allowing precise molecular modeling, aided by complex calculations related to molecular dynamics, property prediction, and virtual screening of compounds (Silva et al., 2022).

Artificial intelligence, allied with data science and machine learning algorithms are an asset to recognize molecular features and establish correlations within large data sets, helping to identify structure-activity relationships and predict biological activities (Shi et al., 2023). Together with this, *in silico* simulations are progressively being used for the investigation of complex molecular interactions in a virtual environment (Ogawa et al., 2023). Combined with the laboratory tests to which the extracts are submitted, it is possible to explore interactions between compounds and biological targets, study thermodynamic and kinetic properties, as well as predict the stability and toxicity of potential candidates from natural products (Gaudêncio et al., 2023).

Additionally, instrumental development aiming to enhance detection sensitivity, automation, and precision is also of critical importance to accelerate natural products investigations. While the natural products field stands to benefit significantly from these technological advancements, classical approaches will remain essential for confirming predictions and validating findings. By recognizing the complementary nature of these methodologies, both fields should progress side by side, leveraging their respective strengths.

Besides the essential techniques and methodologies commonly used and needed for laboratory scientific investigations, the discussion and implementation of public policies that emphasize environmental preservation are also essential. By conducting research in a sustainable manner, developing countries have the opportunity not only to unlock the potential of their biodiversity, but also set an example for the world. Usually, multi-omics investigations are done with the aid of the knowledge acquired from model plants. However, applying or translating this information to other plant species, especially those from wild environments and diverse biomes, is challenging. Therefore, the development of comprehensive databases towards the establishment of new model plants native to those environments would not only contribute to the future studies of wild plant species, but would also leverage the programs for biodiversity protection. Within the realm of Brazilian biodiversity, countless avenues remain unexplored, offering vast opportunities for scientific investigation, biodiversity protection, and valoration. This is also supported by the knowledge in biosynthesis, metabolic engineering, medicinal chemistry, and green chemistry, which has been and will be increasingly essential in the field of synthetic biology and the sustainable production of natural products of high biological and economic value.

Author contributions

HM-R and AP performed the survey of compounds isolated and organisms studied. HM-R, AP, PB, RV, MF, SS, LD, LG, AN, TS-M, MV, and RP wrote the manuscript. AA, AP, IC-G, AC, DS, MF, and VS reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fntpr.2023.1252092/full#supplementary-material

References

Aichler, M., and Walch, A. (2015). MALDI Imaging mass spectrometry: current frontiers and perspectives in pathology research and practice. *Lab. Invest.* 95, 422–431. doi:10.1038/labinvest.2014.156

Alécio, A. C., da Silva Bolzani, V., Young, M. C., Kato, M. J., and Furlan, M. (1998). Antifungal amide from leaves of Piper hispidum. *J. Nat. Prod.* 61, 637–639. doi:10.1021/np9703656

Altei, W. F., Picchi, D. G., Abissi, B. M., Giesel, G. M., Flausino, O., Jr, Reboud-Ravaux, M., et al. (2014). Jatrophidin I, a cyclic peptide from Brazilian Jatropha curcas L. isolation, characterization, conformational studies and biological activity. *Phytochemistry* 107, 91–96. doi:10.1016/j.phytochem.2014.08.006

Alves, T. B., Souza-Moreira, T. M., Valentini, S. R., Zanelli, C. F., and Furlan, M. (2018). Friedelin in Maytenus ilicifolia Is Produced by Friedelin Synthase Isoforms. *Molecules* 23, 700. doi:10.3390/molecules23030700

Aly, A. H., Debbab, A., Kjer, J., and Proksch, P. (2010). Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. *Fungal Divers* 41, 1–16. doi:10.1007/s13225-010-0034-4

Aly, A. H., Debbab, A., and Proksch, P. (2011). Fungal endophytes: unique plant inhabitants with great promises. *Appl. Microbiol. Biotechnol.* 90, 1829–1845. doi:10.1007/s00253-011-3270-y

Andrade, T. J., Somensi, A. H., Lopes, M. N., Araujo, A. R., Jaspars, M., and Silva, D. H. (2013). Identification of quinolactin alkaloids from fungi associated to Brazilian red algae Dichotomaria marginata by LC-PDA-MS and LC-MSn. *Planta Med* 79, 1351868. doi:10.1055/s-0033-1351868

Arwa, P. S., Zeraik, M. L., Ximenes, V. F., da Fonseca, L. M., Bolzani, V. S., and Siqueira Silva, D. H. (2015). Redox-active biflavonoids from Garcinia brasiliensis as inhibitors of neutrophil oxidative burst and human erythrocyte membrane damage. *J. Ethnopharmacol.* 174, 410–418. doi:10.1016/j.jep.2015.08.041

Batista, A., Batista, J., Jr, Souza-Moreira, T., Valentini, S., Kato, M., Zanelli, C., et al. (2018). Biosynthetic Insights into p-Hydroxybenzoic Acid-Derived Benzopyrans in Piper gaudichaudianum. *J. Braz. Chem. Soc.* 29 (5), 20170238. doi:10.21577/0103-5053.20170238

Batista, A. N. L., Santos-Pinto, J. R. A. D., Batista, J. M., Jr, Souza-Moreira, T. M., Santoni, M. M., Zanelli, C. F., et al. (2017). The Combined Use of Proteomics and Transcriptomics Reveals a Complex Secondary Metabolite Network in Peperomia obtusifolia. *J. Nat. Prod.* 80, 1275–1286. doi:10.1021/acs.jnatprod.6b00827

Batista, J. M., Jr, Lopes, A. A., Ambrósio, D. L., Regasini, L. O., Kato, M. J., Bolzani, V. S., et al. (2008). Natural chromenes and chromene derivatives as potential anti-trypanosomal agents. *Biol. Pharm. Bull.* 31, 538–540. doi:10.1248/bpb.31.538

Benevides, P. J., Young, M. C., Giesbrecht, A. M., Roque, N. F., and Bolzani, V. S. (2001). Antifungal polysulphides from Petiveria alliacea L. *Phytochemistry* 57, 743–747. doi:10.1016/s0031-9422(01)00079-6

Bicalho, K. U., Santoni, M. M., Arendt, P., Zanelli, C. F., Furlan, M., Goossens, A., et al. (2019). CYP712K4 Catalyzes the C-29 Oxidation of Friedelin in the Maytenus ilicifolia Quinone Methide Triterpenoid Biosynthesis Pathway. *Plant Cell Physiol* 60, 2510–2522. doi:10.1093/pcp/pcz144

Bobey, A. F., Pinto, M. E. F., Cilli, E. M., Lopes, N. P., and Bolzani, V. S. (2018). A Cyclotide Isolated from Noisettia orchidiflora (Violaceae). *Planta Med* 84, 947–952. doi:10.1055/a-0632-2204

Bode, H. B., Bethe, B., Höfs, R., and Zeeck, A. (2002). Big effects from small changes: possible ways to explore nature's chemical diversity. *Chembiochem* 3, 619–627. doi:10.1002/1439-7633(20020703)3:7<619:aid-cbic619>3.0.co;2-9

Bueno, P. C. P., and Lopes, N. P. (2020). Metabolomics to Characterize Adaptive and Signaling Responses in Legume Crops under Abiotic Stresses. *ACS Omega* 5, 1752–1763. doi:10.1021/acsomega.9b03668

Bueno, P. C. P., Pereira, F. M. V., Torres, R. B., and Cavalheiro, A. J. (2015). Development of a comprehensive method for analyzing clerodane-type diterpenes and phenolic compounds from Casearia sylvestris Swartz (Salicaceae) based on ultra high performance liquid chromatography combined with chemometric tools. *J. Sep. Sci.* 38, 1649–1656. doi:10.1002/jssc.201401421

Buffa Filho, W., Corsino, J., Bolzani, S. V., Furlan, M., Pereira, A. M. S., and França, S. C. (2002). Quantitative determination of cytotoxic-*Friedo-nor*-oleanane derivatives from five morphological types of*Maytenus ilicifolia*(celastraceae) by reverse-phase high-performance liquid chromatography: QUANTITATIVE DETERMINATION OF CYTOTOXIC DITERPENOIDS. *Phytochem. Anal.* 13, 75–78. doi:10.1002/pca.626

Calado, M. L., Silva, J., Alves, C., Susano, P., Santos, D., Alves, J., et al. (2021). Marine endophytic fungi associated with Halopteris scoparia (Linnaeus) Sauvageau as producers of bioactive secondary metabolites with potential dermocosmetic application. *PLoS One* 16, e0250954. doi:10.1371/journal.pone.0250954

Cappello, E., and Nieri, P. (2021). From Life in the Sea to the Clinic: the Marine Drugs Approved and under Clinical Trial. *Life* 11, 1390. doi:10.3390/life11121390

Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2021). Marine Natural Products. *Nat. Prod. Rep.* 38, 362–413. doi:10.1039/d0np00089b

Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2023). Marine natural products. *Nat. Prod. Rep.* 40, 275–325. doi:10.1039/d2np00083k

Cevatemre, B., Erkısa, M., Aztopal, N., Karakas, D., Alper, P., Tsimplouli, C., et al. (2018). A promising natural product, pristimerin, results in cytotoxicity against breast cancer stem cells *in vitro* and xenografts *in vivo* through apoptosis and an incomplete autopaghy in breast cancer. *Pharmacol. Res.* 129, 500–514. doi:10.1016/j.phrs.2017.11.027

Chapla, V. M., Zeraik, M. L., Ximenes, V. F., Zanardi, L. M., Lopes, M. N., Cavalheiro, A. J., et al. (2014). Bioactive secondary metabolites from Phomopsis sp., an endophytic fungus from Senna spectabilis. *Molecules* 19, 6597–6608. doi:10.3390/molecules19056597

Corsino, J., de Carvalho, P. R., Kato, M. J., Latorre, L. R., Oliveira, O. M., Araújo, A. R., et al. (2000). Biosynthesis of friedelane and quinonemethide triterpenoids is compartmentalized in Maytenus aquifolium and Salacia campestris. *Phytochemistry* 55, 741–748. doi:10.1016/s0031-9422(00)00285-5

Costa, P. M., Ferreira, P. M. P., Bolzani, V. S., Furlan, M., Corsino, J., de Moraes, M. O., et al. (2008). Antiproliferative activity of pristimerin isolated from Maytenus ilicifolia (Celastraceae) in human HL-60 cells. *Toxicol.* In Vitro 22, 854–863. doi:10.1016/j.tiv.2008.01.003

Cotinguiba, F., Regasini, L. O., da Silva Bolzani, V., Debonsi, H. M., Duó Passerini, G., Cicarelli, R. M. B., et al. (2009). Piperamides and their derivatives as potential anti-trypanosomal agents. *Med. Chem. Res.* 18, 703–711. doi:10.1007/s00044-008-9161-9

Craik, D. J., Daly, N. L., Bond, T., and Waine, C. (1999). Plant cyclotides: A unique family of cyclic and knotted proteins that defines the cyclic cystine knot structural motif. *J. Mol. Biol.* 294, 1327–1336. doi:10.1006/jmbi.1999.3383

Craik, D. J., Lee, M.-H., Rehm, F. B. H., Tombling, B., Doffek, B., and Peacock, H. (2018). Ribosomally-synthesised cyclic peptides from plants as drug leads and pharmaceutical scaffolds. *Bioorg. Med. Chem.* 26, 2727–2737. doi:10.1016/j.bmc.2017.08.005

Croteau, R., Ketchum, R. E. B., Long, R. M., Kaspera, R., and Wildung, M. R. (2006). Taxol biosynthesis and molecular genetics. *Phytochem. Rev.* 5, 75–97. doi:10.1007/s11101-005-3748-2

Dametto, A. C., Agustoni, D., Moreira, T. F., Plaza, C. V., Prieto, A. M., Silva, T. G. A., et al. (2017). Chemical composition and *in vitro* chemoprevention assessment of Eugenia jambolana Lam. (Myrtaceae) fruits and leaves. *J. Funct. Foods* 36, 490–502. doi:10.1016/j.jff.2017.07.013

da Silva Mota, J., Leite, A. C., Batista Junior, J. M., Noelí López, S., Luz Ambrósio, D., Duó Passerini, G., et al. (2009). *In vitro* trypanocidal activity of phenolic derivatives from Peperomia obtusifolia. *Planta Med* 75, 620–623. doi:10.1055/s-0029-1185364

da Saúde, M. (2023). Plantas Medicinais de Interesse do SUS - Renisus. Available at: https://www.gov.br/saude/pt-br/composicao/sectics/daf/pnpmf/ppnpmf/renisus (Accessed May, 2023).

de JASAndrade, T., Somensi, A., Araújo, A. R., Jaspars, M., and Silva, D. H. S. (2015). Penicillium chrysogenum, an endophyte from Padina gymnospora, as a source of diketopiperazines, peptides and griseofulvin. *Planta Med* 81, PL5. doi:10.1055/s-0035-1556313

de JASAndrade, T., Somensi, A., Lopes, M. N., Araújo, A. R., Jaspars, M., and Silva, D. H. S. (2014). Citrinadin A derivatives from Penicillium citrinum, an endophyte from the marine red alga Dichotomaria marginata. *Planta Med* 80, PC37. doi:10.1055/s-0034-1382419

de Oliveira, C. M., Silva, G. H., Regasini, L. O., Flausino, O., López, S. N., Abissi, B. M., et al. (2011). Xylarenones C-E from an endophytic fungus isolated from Alibertia macrophylla. *J. Nat. Prod.* 74, 1353–1357. doi:10.1021/np1005983

de Oliveira Filho, J. W. G., Andrade, T. J. A. D. S., de Lima, R. M. T., Dos Reis, A. C., Silva, D. H. S., Santos, J. V. O., et al. (2021). Citrinin against breast cancer: A cytogenotoxicological study. *Phytother. Res.* 35, 504–516. doi:10.1002/ptr.6830

de Oliveira Filho, J. W. G., Andrade, T. J. A. D. S., de Lima, R. M. T., Silva, D. H. S., Dos Reis, A. C., Santos, J. V. O., et al. (2022). Cytogenotoxic evaluation of the acetonitrile extract, citrinin and dicitrinin-A from Penicillium citrinum. *Drug Chem. Toxicol.* 45, 688–697. doi:10.1080/01480545.2020.1769642

de Oliveira Filho, J. W. G., Dos Santos Andrade, T. J. A., De Lima, R. M. T., Dos Reis, A. C., Hameed, A., Santos, J. V. O., et al. (2020). Antitumor effects of citrinin in an animal model of Sarcoma 180 via cytogenetic mechanisms. *Cell. Mol. Biol.* 66, 205–215.

dos Santos, A. G., Ferreira, P. M. P., Vieira Júnior, G. M., Perez, C. C., Gomes Tininis, A., Silva, G. H., et al. (2010a). Casearin X, its degradation product and other clerodane diterpenes from leaves of Casearia sylvestris: evaluation of cytotoxicity against normal and tumor human cells. *Chem. Biodivers.* 7, 205–215. doi:10.1002/cbdv.200800342

Dos Santos, V. A., Dos Santos, D. P., Castro-Gamboa, I., Zanoni, M. V. B., and Furlan, M. (2010b). Evaluation of antioxidant capacity and synergistic associations of quinonemethide triterpenes and phenolic substances from Maytenus ilicifolia (Celastraceae). *Molecules* 15, 6956–6973. doi:10.3390/molecules15106956

Dos Santos, V. A. F. F. M., Leite, K. M., da Costa Siqueira, M., Regasini, L. O., Martinez, I., Nogueira, C. T., et al. (2013). Antiprotozoal activity of quinonemethide triterpenes from Maytenus ilicifolia (Celastraceae). *Molecules* 18, 1053–1062. doi:10.3390/molecules18011053

El-Bondkly, E. A. M., El-Bondkly, A. A. M., and El-Bondkly, A. A. M. (2021). Marine endophytic fungal metabolites: A whole new world of pharmaceutical therapy exploration. *Heliyon* 7, e06362. doi:10.1016/j.heliyon.2021.e06362

Endo, A. (2008). A gift from nature: the birth of the statins. *Nat. Med.* 14, 1050–1052. doi:10.1038/nm1008-1050

Fapesp, A. (2013). Brazilian database of natural products receives international recognition. Agência FAPESP. Available at: https://agencia.fapesp.br/brazilian-database-of-natural-products-receives-international-recognition/17094/(Accessed May 7, 2023).

Felix Alves, N., Castro Ribeiro, D., Mannochio-Russo, H., Silva Queiroz, S. A., Zocolo, G. J., da Silva Bolzani, V., et al. (2023). Advanced Glycation End Products Inhibitors

from Talisia esculenta. *Rev. Virtual Quím* 15 (3), 1–7. doi:10.21577/1984-6835.20230019

Fernandes, D. C., Regasini, L. O., Vellosa, J. C. R., Pauletti, P. M., Castro-Gamboa, I., Bolzani, V. S., et al. (2008). Myeloperoxidase inhibitory and radical scavenging activities of flavones from Pterogyne nitens. *Chem. Pharm. Bull.* 56, 723–726. doi:10.1248/cpb.56.723

Fernández-Bobey, A., Pinto, M. E. F., de Almeida, L. C., de Souza, B. M., Dias, N. B., de Paula-Souza, J., et al. (2022). Cytotoxic Cyclotides from *Anchietea pyrifolia*, a South American Plant Species. *J. Nat. Prod.* 85, 2127–2134. doi:10.1021/acs.jnatprod.1c01129

Fernández-de Gortari, E., García-Jacas, C. R., Martinez-Mayorga, K., and Medina-Franco, J. L. (2017). Database fingerprint (DFP): an approach to represent molecular databases. *J. Cheminform.* 9, 9. doi:10.1186/s13321-017-0195-1

Ferreira, V. G., Leme, G. M., Cavalheiro, A. J., and Funari, C. S. (2016). Online Extraction Coupled to Liquid Chromatography Analysis (OLE-LC): eliminating Traditional Sample Preparation Steps in the Investigation of Solid Complex Matrices. *Anal. Chem.* 88, 8421–8427. doi:10.1021/acs.analchem.6b02388

Flausino, O., Jr, Santos, L. de A., Verli, H., Pereira, A. M., Bolzani, V. da S., and Nunes-de-Souza, R. L. (2007). Anxiolytic effects of erythrinian alkaloids from Erythrina mulungu. *J. Nat. Prod.* 70, 48–53. doi:10.1021/np060254j

Fraige, K., Arrua, R. D., Sutton, A. T., Funari, C. S., Cavalheiro, A. J., Hilder, E. F., et al. (2019). Using natural deep eutectic solvents for the extraction of metabolites in Byrsonima intermedia leaves. *J. Sep. Sci.* 42, 591–597. doi:10.1002/jssc.201800905

Freire, R. T., and Castro-Gamboa, I. (2015). *Method of analysis' pattern recognition and computer program*. World Patent.

Freitas, L. de, Valli, M., Dametto, A. C., Pennacchi, P. C., Andricopulo, A. D., Maria-Engler, S. S., et al. (2020). Advanced Glycation End Product Inhibition by Alkaloids from Ocotea paranapiacabensis for the Prevention of Skin Aging. *J. Nat. Prod.* 83, 649–656. doi:10.1021/acs.jnatprod.9b01083

Funari, C. S., Carneiro, R. L., Andrade, A. M., Hilder, E. F., and Cavalheiro, A. J. (2014). Green chromatographic fingerprinting: an environmentally friendly approach for the development of separation methods for fingerprinting complex matrices. *J. Sep. Sci.* 37, 37–44. doi:10.1002/jssc.201300955

Funari, C. S., Carneiro, R. L., Creese, M. E., Leme, G. M., Cavalheiro, A. J., and Hilder, E. F. (2016). On Track for a Truly Green Propolis—Fingerprinting Propolis Samples from Seven Countries by Means of a Fully Green Approach. *ACS Sustainable Chem. Eng.* 4, 7110–7117. doi:10.1021/acssuschemeng.6b02005

Funari, C. S., Carneiro, R. L., Khandagale, M. M., Cavalheiro, A. J., and Hilder, E. F. (2015). Acetone as a greener alternative to acetonitrile in liquid chromatographic fingerprinting. *J. Sep. Sci.* 38, 1458–1465. doi:10.1002/jssc.201401324

Funari, C. S., Sutton, A. T., Carneiro, R. L., Fraige, K., Cavalheiro, A. J., da Silva Bolzani, V., et al. (2019). Natural deep eutectic solvents and aqueous solutions as an alternative extraction media for propolis. *Food Res. Int.* 125, 108559. doi:10.1016/j.foodres.2019.108559

Gaudêncio, S. P., Bayram, E., Lukić Bilela, L., Cueto, M., Díaz-Marrero, A. R., Haznedaroglu, B. Z., et al. (2023). Advanced Methods for Natural Products Discovery: bioactivity Screening, Dereplication, Metabolomics Profiling, Genomic Sequencing, Databases and Informatic Tools, and Structure Elucidation. *Mar. Drugs* 21, 308. doi:10.3390/md21050308

Gerlach, S. L., Göransson, U., Kaas, Q., Craik, D. J., Mondal, D., and Gruber, C. W. (2013). A systematic approach to document cyclotide distribution in plant species from genomic, transcriptomic, and peptidomic analysis. *Biopolymers* 100, 433–437. doi:10.1002/bip.22258

Gubiani, J. R., Nogueira, C. R., Pereira, M. D. P., Young, M. C. M., Ferreira, P. M. P., de Moraes, M. O., et al. (2016). Rearranged Sesquiterpenes and Branched Polyketides Produced by the Endophyte Camarops sp. *Phytochem. Lett.* 17, 251–257. doi:10.1016/j.phytol.2016.08.007

Gubiani, J. R., Zeraik, M. L., Oliveira, C. M., Ximenes, V. F., Nogueira, C. R., Fonseca, L. M., et al. (2014). Biologically active eremophilane-type sesquiterpenes from Camarops sp., an endophytic fungus isolated from Alibertia macrophylla. *J. Nat. Prod.* 77, 668–672. doi:10.1021/np400825s

Gunatilaka, A. A. L. (2006). Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J. Nat. Prod.* 69, 509–526. doi:10.1021/np058128n

Harvey, A. L., Edrada-Ebel, R., and Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nat. Rev. Drug Discov.* 14, 111–129. doi:10.1038/nrd4510

Horton, D. A., Bourne, G. T., and Smythe, M. L. (2002). Exploring privileged structures: the combinatorial synthesis of cyclic peptides. *J. Comput. Aided Mol. Des.* 16, 415–430. doi:10.1023/a:1020863921840

Hu, G., and Qiu, M. (2023). Machine learning-assisted structure annotation of natural products based on MS and NMR data. *Nat. Prod. Rep.* 2023, 25. doi:10.1039/d3np00025g

Jardim, A. C. G., Igloi, Z., Shimizu, J. F., Santos, V. A. F. F. M., Felippe, L. G., Mazzeu, B. F., et al. (2015). Natural compounds isolated from Brazilian plants are potent inhibitors of hepatitis C virus replication *in vitro*. *Antiviral Res* 115, 39–47. doi:10.1016/j.antiviral.2014.12.018

Jeon, J., Kang, S., and Kim, H. U. (2021). Predicting biochemical and physiological effects of natural products from molecular structures using machine learning. *Nat. Prod. Rep.* 38, 1954–1966. doi:10.1039/d1np00016k

Joo, S. H. (2012). Cyclic peptides as therapeutic agents and biochemical tools. *Biomol. Ther.* 20, 19–26. doi:10.4062/biomolther.2012.20.1.019

Kato, M. J., and Furlan, M. (2007). Chemistry and evolution of the Piperaceae. *J. Macromol. Sci. Part A Pure Appl. Chem.* 79, 529–538. doi:10.1351/pac200779040529

Kim, H. W., Wang, M., Leber, C. A., Nothias, L.-F., Reher, R., Kang, K. B., et al. (2021). NPClassifier: A Deep Neural Network-Based Structural Classification Tool for Natural Products. *J. Nat. Prod.* 84, 2795–2807. doi:10.1021/acs.jnatprod.1c00399

Leite, A. C., Lopes, A. A., Kato, M. J., Bolzani, V. S., and Furlan, M. (2007). Biosynthetic origin of the isoprene units in chromenes of Piper aduncum (Piperaceae). *J. Braz. Chem. Soc.* 18, 1500–1503. doi:10.1590/s0103-50532007000800008

Leme, G. M., Cacciola, F., Donato, P., Cavalheiro, A. J., Dugo, P., and Mondello, L. (2014). Continuous vs. segmented second-dimension system gradients for comprehensive two-dimensional liquid chromatography of sugarcane (Saccharum spp.). *Anal. Bioanal. Chem.* 406, 4315–4324. doi:10.1007/s00216-014-7786-8

Lopes, A. A., Baldoqui, D. C., López, S. N., Kato, M. J., Bolzani, V. S., and Furlan, M. (2007). Biosynthetic origins of the isoprene units of gaudichaudianic acid in Piper gaudichaudianum (Piperaceae). *Phytochemistry* 68, 2053–2058. doi:10.1016/j.phytochem.2007.04.025

López, S. N., Lopes, A. A., Batista, J. M., Jr, Flausino, O., Jr, Bolzani, V., da, S., Kato, M. J., et al. (2010). Geranylation of benzoic acid derivatives by enzymatic extracts from Piper crassinervium (Piperaceae). *Bioresour. Technol.* 101, 4251–4260. doi:10.1016/j.biortech.2010.01.041

Lo, Y.-C., Rensi, S. E., Torng, W., and Altman, R. B. (2018). Machine learning in chemoinformatics and drug discovery. *Drug Discov. Today* 23, 1538–1546. doi:10.1016/j.drudis.2018.05.010

Mannochio-Russo, H., Bueno, P. C. P., Bauermeister, A., de Almeida, R. F., Dorrestein, P. C., Cavalheiro, A. J., et al. (2020). 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. *J. Nat. Prod.* 83, 3239–3249. doi:10.1021/acs.jnatprod.0c00495

Mannochio-Russo, H., de Almeida, R. F., Nunes, W. D. G., Bueno, P. C. P., Caraballo-Rodríguez, A. M., Bauermeister, A., et al. (2022). Untargeted metabolomics sheds light on the diversity of major classes of secondary metabolites in the Malpighiaceae botanical family. *Front. Plant Sci.* 13, 854842. doi:10.3389/fpls.2022.854842

Mannochio-Russo, H., Nunes, W. D. G., Almeida, R. F., Albernaz, L. C., Espindola, L. S., and Bolzani, V. S. (2023). Old Meets New: mass Spectrometry-Based Untargeted Metabolomics Reveals Unusual Larvicidal Nitropropanoyl Glycosides from the Leaves of Heteropterys umbellata. *J. Nat. Prod.* 86, 621–632. doi:10.1021/acs.jnatprod.2c00788

Marine Pharmacology (2023). Approved Marine Drugs. Available at: https://www.marinepharmacology.org/approved (Accessed March 28, 2023).

Mazzeu, B. F., Souza-Moreira, T. M., Oliveira, A. A., Remlinger, M., Felippe, L. G., Valentini, S. R., et al. (2021). The Methionine 549 and Leucine 552 Residues of Friedelin Synthase from Maytenus ilicifolia Are Important for Substrate Binding Specificity. *Molecules* 26, 6806. doi:10.3390/molecules26226806

Medina-Franco, J. L. (2013). Advances in computational approaches for drug discovery based on natural products. *Revista latinoamericana de química*. Available at: https://scielo.org.mx/scielo.php?script=sci_arttext&pid=S0370-59432013000200003 (Accessed May 7, 2023).

Medina-Franco, J. L., and Maggiora, G. M. (2013). "MOLECULAR SIMILARITY ANALYSIS," in *Chemoinformatics for Drug Discovery* (Hoboken, NJ: John Wiley &Sons, Inc), 343–399.

Medina, R. P., Araujo, A. R., Andersen, R. J., Soares, M. A., Silva, F. de A., and Silva, D. H. S. (2019a). Aromatic compounds produced by endophytic fungi isolated from red alga Asparagopsis taxiformis - Falkenbergia stage. *Nat. Prod. Res.* 33, 443–446. doi:10.1080/14786419.2018.1455037

Medina, R. P., Araujo, A. R., Batista, J. M.,, Jr, Cardoso, C. L., Seidl, C., Vilela, A. F. L., et al. (2019b). Botryane terpenoids produced by Nemania bipapillata, an endophytic fungus isolated from red alga Asparagopsis taxiformis - Falkenbergia stage. *Sci. Rep.* 9, 12318. doi:10.1038/s41598-019-48655-7

MMA (2023). Ministério do Meio Ambiente. Available at: www.mma.gov.br/portalbio (Accessed May, 2023).

Modi, S., Hughes, M., Garrow, A., and White, A. (2012). The value of in silico chemistry in the safety assessment of chemicals in the consumer goods and pharmaceutical industries. *Drug Discov. Today* 17, 135–142. doi:10.1016/j.drudis.2011.10.022

Monteiro, A. F., Righetto, G. M., Simões, L. V., Almeida, L. C. de, Costa-Lotufo, L. V., Camargo, I. L. B. C., et al. (2019). Oxidative functionalization of a halimane diterpenoid achieved by fungal transformation. *Bioorg. Chem.* 86, 550–556. doi:10.1016/j.bioorg.2019.02.021

Monteiro, A. F., Seidl, C., Severino, V. G. P., Cardoso, C. L., and Castro-Gamboa, I. (2017). Biotransformation of labdane and halimane diterpenoids by two filamentous fungi strains. *R Soc Open Sci* 4, 170854. doi:10.1098/rsos.170854

Morandim, A. de A., Bergamo, D. C. B., Kato, M. J., Cavalheiro, A. J., Bolzani, V. S., and Furlan, M. (2005). Circadian rhythm of anti-fungal prenylated chromene in leaves of Piper aduncum. *Phytochem. Anal.* 16, 282–286. doi:10.1002/pca.843

Navickiene, H. M., Alécio, A. C., Kato, M. J., Bolzani, V. D., Young, M. C., Cavalheiro, A. J., et al. (2000). Antifungal amides from Piper hispidum and Piper tuberculatum. *Phytochemistry* 55, 621–626. doi:10.1016/s0031-9422(00)00226-0

Newman, D. J., and Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* 83, 770–803. doi:10.1021/acs.jnatprod.9b01285

Noble, R. L. (1990). The discovery of the vinca alkaloids--chemotherapeutic agents against cancer. *Biochem. Cell Biol.* 68, 1344–1351. doi:10.1139/o90-197

Ntie-Kang, F., Telukunta, K. K., Döring, K., Simoben, C. V., A Moumbock, A. F., Malange, Y. I., et al. (2017). NANPDB: A Resource for Natural Products from Northern African Sources. *J. Nat. Prod.* 80, 2067–2076. doi:10.1021/acs.jnatprod.7b00283

Ogawa, K., Sakamoto, D., and Hosoki, R. (2023). Computer Science Technology in Natural Products Research: A Review of Its Applications and Implications. *Chem. Pharm. Bull.* 71, 486–494. doi:10.1248/cpb.c23-00039

Oliveira, A. L., Viegas, M. F., da Silva, S. L., Soares, A. M., Ramos, M. J., and Fernandes, P. A. (2022). The chemistry of snake venom and its medicinal potential. *Nat Rev Chem* 6, 451–469. doi:10.1038/s41570-022-00393-7

Oliveira, C. M., Regasini, L. O., Silva, G. H., Pfenning, L. H., Young, M. C. M., Berlinck, R. G. S., et al. (2011). Dihydroisocoumarins produced by Xylaria sp. and Penicillium sp., endophytic fungi associated with Piper aduncum and Alibertia macrophylla. *Phytochem. Lett.* 4, 93–96. doi:10.1016/j.phytol.2010.11.003

Oliveira, C. M., Silva, G. H., Regasini, L. O., Zanardi, L. M., Evangelista, A. H., Young, M. C. M., et al. (2009). Bioactive metabolites produced by Penicillium sp. 1 and sp. 2, two endophytes associated with Alibertia macrophylla (Rubiaceae). *Z. Naturforsch. C* 64, 824–830. doi:10.1515/znc-2009-11-1212

Oliveira, A. L. L., Felício, R., and Debonsi, H. M. (2012). Marine natural products: chemical and biological potential of seaweeds and their endophytic fungi. *Rev. Bras. Farmacogn.* 22, 906–920. doi:10.1590/s0102-695x2012005000083

Passalacqua, T. G., Dutra, L. A., de Almeida, L., Velásquez, A. M. A., Torres, F. A. E., Yamasaki, P. R., et al. (2015a). Synthesis and evaluation of novel prenylated chalcone derivatives as anti-leishmanial and anti-trypanosomal compounds. *Bioorg. Med. Chem. Lett.* 25, 3342–3345. doi:10.1016/j.bmcl.2015.05.072

Passalacqua, T. G., Torres, F. A. E., Nogueira, C. T., de Almeida, L., Del Cistia, M. L., dos Santos, M. B., et al. (2015b). The 2',4'-dihydroxychalcone could be explored to develop new inhibitors against the glycerol-3-phosphate dehydrogenase from Leishmania species. *Bioorg. Med. Chem. Lett.* 25, 3564–3568. doi:10.1016/j.bmcl.2015.06.085

Pauletti, P. M., Araújo, A. R., Young, M. C., Giesbrecht, A. M., and Bolzani, V. D. (2000). nor-Lignans from the leaves of Styrax ferrugineus (Styracaceae) with antibacterial and antifungal activity. *Phytochemistry* 55, 597–601. doi:10.1016/s0031-9422(00)00225-9

Pereira, F., and Aires-de-Sousa, J. (2018). Computational Methodologies in the Exploration of Marine Natural Product Leads. *Mar. Drugs* 16, 236. doi:10.3390/md16070236

Pilon, A. C., Gu, H., Raftery, D., Bolzani, V. S., Lopes, N. P., Castro-Gamboa, I., et al. (2019). Mass Spectral Similarity Networking and Gas-Phase Fragmentation Reactions in the Structural Analysis of Flavonoid Glycoconjugates. *Anal. Chem.* 91, 10413–10423. doi:10.1021/acs.analchem.8b05479

Pilon, A. C., Valli, M., Dametto, A. C., Pinto, M. E. F., Freire, R. T., Castro-Gamboa, I., et al. (2017). NuBBEDB: an updated database to uncover chemical and biological information from Brazilian biodiversity. *Sci. Rep.* 7, 7215. doi:10.1038/s41598-017-07451-x

Pilon, A., Selegato, D., Fernandes, R., Bueno, P., Pinho, D., Carnevale Neto, F., et al. (2020). Metabolômica De Plantas: Métodos E Desafios. *Quim. Nova* 43 (3), 20170499. doi:10.21577/0100-4042.20170499

Pilon, A., Vieira, N., Amaral, J., Monteiro, A., Silva, R., Spíndola, L., et al. (2021). Redes Moleculares: uma Análise Sobre Anotações E Descoberta De Novos Ativos. *Quim. Nova.* 2021, 20170777. doi:10.21577/0100-4042.20170777

Pinedo-Rivilla, C., Aleu, J., and Durán-Patrón, R. (2022). Cryptic Metabolites from Marine-Derived Microorganisms Using OSMAC and Epigenetic Approaches. *Mar. Drugs* 20, 84. doi:10.3390/md20020084

Pinto, M. E. F., Batista, J. M., Jr, Koehbach, J., Gaur, P., Sharma, A., Nakabashi, M., et al. (2015). Ribifolin, an orbitide from Jatropha ribifolia, and its potential antimalarial activity. *J. Nat. Prod.* 78, 374–380. doi:10.1021/np5007668

Pinto, M. E. F., Chan, L. Y., Koehbach, J., Devi, S., Gründemann, C., Gruber, C. W., et al. (2021). Cyclotides from Brazilian *Palicourea sessilis* and Their Effects on Human Lymphocytes. *J. Nat. Prod.* 84, 81–90. doi:10.1021/acs.jnatprod.0c01069

Pinto, M. E. F., Najas, J. Z. G., Magalhães, L. G., Bobey, A. F., Mendonça, J. N., Lopes, N. P., et al. (2018). Inhibition of Breast Cancer Cell Migration by Cyclotides Isolated from Pombalia calceolaria. *J. Nat. Prod.* 81, 1203–1208. doi:10.1021/acs.jnatprod.7b00969

Pivatto, M., Baccini, L. R., Sharma, A., Nakabashi, M., Danuello, A., Viegas Júnior, C., et al. (2014). Antimalarial Activity of Piperidine Alkaloids fromSenna spectabilisand Semisynthetic Derivatives. *J. Braz. Chem. Soc.* 25 (10), 20140195. doi:10.5935/0103-5053.20140195

Queiroz, S. A. S., Pinto, M. E. F., Bobey, A. F., Russo, H. M., Batista, A. N. L., Batista, J. M., Jr, et al. (2020). Diterpenoids with inhibitory activity of nitrite production from Croton floribundus. *J. Ethnopharmacol.* 249, 112320. doi:10.1016/j.jep.2019.112320

Ramalho, S. D., Pinto, M. E. F., Ferreira, D., and Bolzani, V. S. (2018a). Biologically Active Orbitides from the Euphorbiaceae Family. *Planta Med* 84, 558–567. doi:10.1055/s-0043-122604

Ramalho, S. D., Wang, C. K., King, G. J., Byriel, K. A., Huang, Y.-H., Bolzani, V. S., et al. (2018b). Synthesis, Racemic X-ray Crystallographic, and Permeability Studies of Bioactive Orbitides from Jatropha Species. *J. Nat. Prod.* 81, 2436–2445. doi:10.1021/acs.jnatprod.8b00447

Ramalho, S., Pinto, M. E., Andricopulo, R., Sanches, P., Silveira, E., Cilli, E., et al. (2019). Linear peptide analogues from Jatropha's orbitides promote migration of human neonatal foreskin fibroblasts *in vitro* and collagen deposition. *J. Braz. Chem. Soc.* 30 (10), 20190102. doi:10.21577/0103-5053.20190102

Ribeiro, D., Russo, H., Fraige, K., Zeraik, M., Nogueira, C., da Silva, P., et al. (2021). Bioactive Bioflavonoids from Platonia insignis (Bacuri) Residues as Added Value Compounds. *J. Braz. Chem. Soc.* 32 (4), 20200230. doi:10.21577/0103-5053.20200230

Sabandar, C. W., Ahmat, N., Jaafar, F. M., and Sahidin, I. (2013). Medicinal property, phytochemistry and pharmacology of several Jatropha species (Euphorbiaceae): a review. *Phytochemistry* 85, 7–29. doi:10.1016/j.phytochem.2012.10.009

Saldívar-González, F. I., Valli, M., Andricopulo, A. D., da Silva Bolzani, V., and Medina-Franco, J. L. (2019). Chemical Space and Diversity of the NuBBE Database: A Chemoinformatic Characterization. *J. Chem. Inf. Model.* 59, 74–85. doi:10.1021/acs.jcim.8b00619

Santos, V. A. F. F. M., Regasini, L. O., Nogueira, C. R., Passerini, G. D., Martinez, I., Bolzani, V. S., et al. (2012). Antiprotozoal sesquiterpene pyridine alkaloids from Maytenus ilicifolia. *J. Nat. Prod.* 75, 991–995. doi:10.1021/np300077r

Selegato, D. M., Freire, R. T., Pilon, A. C., Biasetto, C. R., de Oliveira, H. C., de Abreu, L. M., et al. (2019). Improvement of bioactive metabolite production in microbial cultures-A systems approach by OSMAC and deconvolution-based 1 HNMR quantification. *Magn. Reson. Chem.* 57, 458–471. doi:10.1002/mrc.4874

Selegato, D. M., Freire, R. T., Tannús, A., and Castro-Gamboa, I. (2016). New Dereplication Method Applied to NMR-Based Metabolomics on DifferentFusariumSpecies Isolated from Rhizosphere ofSenna spectabilis. *J. Braz. Chem. Soc.* 27 (8), 20160139. doi:10.5935/0103-5053.20160139

Shi, Y.-F., Yang, Z.-X., Ma, S., Kang, P.-L., Shang, C., Hu, P., et al. (2023). Machine Learning for Chemistry: basics and Applications. *Proc. Est. Acad. Sci. Eng.* 2023, 013. doi:10.1016/j.eng.2023.04.013

Silva, D. H. S., Mannochio-Russo, H., Lago, J. H. G., Bueno, P. C. P., Medina, R. P., Bolzani, V. S., et al. (2022). Bioprospecting as a strategy for conservation and sustainable use of the Brazilian Flora. *Biota Neotrop* 22, e20221356. doi:10.1590/1676-0611-bn-2022-1356

Silva, G. H., de Oliveira, C. M., Teles, H. L., Pauletti, P. M., Castro-Gamboa, I., Silva, D. H. S., et al. (2010). Sesquiterpenes from Xylaria sp., an endophytic fungus associated with Piper aduncum (Piperaceae). *Phytochem. Lett.* 3, 164–167. doi:10.1016/j.phytol.2010.07.001

Silva, G. H., Teles, H. L., Trevisan, H. C., Bolzani, V. S., Young, M. C. M., Pfenning, L. H., et al. (2005). New bioactive metabolites produced by Phomopsis cassiae, an endophytic fungus in Cassia spectabilis. *J. Braz. Chem. Soc.* 16, 1463–1466. doi:10.1590/s0103-50532005000800029

Silva, G. H., Teles, H. L., Zanardi, L. M., Marx Young, M. C., Eberlin, M. N., Hadad, R., et al. (2006). Cadinane sesquiterpenoids of Phomopsis cassiae, an endophytic fungus associated with Cassia spectabilis (Leguminosae). *Phytochemistry* 67, 1964–1969. doi:10.1016/j.phytochem.2006.06.004

Silva, G. H., Zeraik, M. L., de Oliveira, C. M., Teles, H. L., Trevisan, H. C., Pfenning, L. H., et al. (2017). Lactone Derivatives Produced by a Phaeoacremonium sp., an Endophytic Fungus from Senna spectabilis. *J. Nat. Prod.* 80, 1674–1678. doi:10.1021/acs.jnatprod.5b00828

Sorokina, M., and Steinbeck, C. (2020). Review on natural products databases: where to find data in 2020. *J. Cheminform.* 12, 20. doi:10.1186/s13321-020-00424-9

Souza-Moreira, T. M., Alves, T. B., Pinheiro, K. A., Felippe, L. G., De Lima, G. M. A., Watanabe, T. F., et al. (2016). Friedelin Synthase from Maytenus ilicifolia: leucine 482 Plays an Essential Role in the Production of the Most Rearranged Pentacyclic Triterpene. *Sci. Rep.* 6, 36858. doi:10.1038/srep36858

Souza-Moreira, T. M., Navarrete, C., Chen, X., Zanelli, C. F., Valentini, S. R., Furlan, M., et al. (2018). Screening of 2A peptides for polycistronic gene expression in yeast. *FEMS Yeast Res* 18. doi:10.1093/femsyr/foy036

Souza, O. A., Ramalhão, V. G. S., Trentin, L. de M., Funari, C. S., Carneiro, R. L., Bolzani, V. S., et al. (2022). Combining natural deep eutectic solvent and microwave irradiation towards the eco-friendly and optimized extraction of bioactive phenolics from Eugenia uniflora L. *Sustainable Chemistry and Pharmacy* 26, 100618. doi:10.1016/j.scp.2022.100618

Stierle, A., Strobel, G., and Stierle, D. (1993). Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. *Science* 260, 214–216. doi:10.1126/science.8097061

Sutton, A. T., Fraige, K., Leme, G. M., da Silva Bolzani, V., Hilder, E. F., Cavalheiro, A. J., et al. (2018). Natural deep eutectic solvents as the major mobile phase components in high-performance liquid chromatography-searching for alternatives to organic solvents. *Anal. Bioanal. Chem.* 410, 3705–3713. doi:10.1007/s00216-018-1027-5

Teixeira, T. R., Santos, G. S. D., Armstrong, L., Colepicolo, P., and Debonsi, H. M. (2019). Antitumor Potential of Seaweed Derived-Endophytic Fungi. *Antibiotics (Basel)* 8, 205. doi:10.3390/antibiotics8040205

Teles, H. L., Hemerly, J. P., Paulettit, P. M., Pandolfi, J. R. C., Araujot, A. R., Valentini, S. R., et al. (2005). Cytotoxic lignans from the stems of Styrax camporum (Styracaceae). *Nat. Prod. Res.* 19, 319–323. doi:10.1080/14786410412331271979

Valli, M., Altei, W., dos Santos, R., de Lucca, E., Jr, Dessoy, M., Pioli, R., et al. (2017). Synthetic analogue of the natural product piperlongumine as a potent inhibitor of breast cancer cell line migration. *J. Braz. Chem. Soc.* 28, 475–484. doi:10.21577/0103-5053.20160303

Valli, M., dos Santos, R. N., Figueira, L. D., Nakajima, C. H., Castro-Gamboa, I., Andricopulo, A. D., et al. (2013). Development of a natural products database from the biodiversity of Brazil. *J. Nat. Prod.* 76, 439–444. doi:10.1021/np3006875

Valli, M., Russo, H. M., and Bolzani, V. S. (2018). The potential contribution of the natural products from Brazilian biodiversity to bioeconomy. *An. Acad. Bras. Cienc.* 90, 763–778. doi:10.1590/0001-3765201820170653

Valli, M., Russo, H. M., Pilon, A. C., Pinto, M. E. F., Dias, N. B., Freire, R. T., et al. (2019a). Computational methods for NMR and MS for structure elucidation II: database resources and advanced methods. *Physical Sciences Reviews* 4. doi:10.1515/psr-2018-0167

Valli, M., Russo, H. M., Pilon, A. C., Pinto, M. E. F., Dias, N. B., Freire, R. T., et al. (2019b). Computational methods for NMR and MS for structure elucidation I: software for basic NMR. *Physical Sciences Reviews* 4. doi:10.1515/psr-2018-0108

Vasques da Silva, R., Navickiene, H. M. D., Kato, M. J., Bolzani, V. S., Méda, C. I., Young, M. C. M., et al. (2002). Antifungal amides from Piper arboreum and Piper tuberculatum. *Phytochemistry* 59, 521–527. doi:10.1016/s0031-9422(01)00431-9

Vieira-Júnior, G. M., Dutra, L. A., Ferreira, P. M. P., de Moraes, M. O., Costa Lotufo, L. V., Pessoa, C. do Ó., et al. (2011). Cytotoxic clerodane diterpenes from Casearia rupestris. *J. Nat. Prod.* 74, 776–781. doi:10.1021/np100840w

Vieira, R., de Sousa, K. A., and Castro-Gamboa, I. (2023a). *Lumios - Label Using Machine in Organic Samples - A Software for Dereplication, Molecular Docking, And Combined Machine and Deep Learning*. doi:10.2139/ssrn.4341603

Vieira, R., de Sousa, K. A., Monteiro, A. F., Pinto, L. S., and Castro-Gamboa, I. (2021). Induction of metabolic variability of the endophytic fungus Xylaria sp. by OSMAC approach and experimental design. *Arch. Microbiol.* 203, 3025–3032. doi:10.1007/s00203-021-02283-w

Vieira, R., dos Santos, A. L. P., Moura, L. H. S., Chapla, V., Bueno, P. C. P., Valli, M., et al. (2023b). *NuBBE: história, avanços e perspectivas em Produtos Naturais da biodiversidade brasileira*. Química de Produtos Naturais do Brasil: trajetória, atualidades e perspectivas.

Villoutreix, B. O., Lagorce, D., Labbé, C. M., Sperandio, O., and Miteva, M. A. (2013). One hundred thousand mouse clicks down the road: selected online resources supporting drug discovery collected over a decade. *Drug Discov. Today* 18, 1081–1089. doi:10.1016/j.drudis.2013.06.013

Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P., and McPhail, A. T. (1971). Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* 93, 2325–2327. doi:10.1021/ja00738a045

Wilkinson, M. D., Dumontier, M., Aalbersberg, I. J. J., Appleton, G., Axton, M., Baak, A., et al. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Sci Data* 3, 160018. doi:10.1038/sdata.2016.18

Zeraik, M. L., Queiroz, E. F., Marcourt, L., Ciclet, O., Castro-Gamboa, I., Silva, D. H. S., et al. (2016). Antioxidants, quinone reductase inducers and acetylcholinesterase inhibitors from Spondias tuberosa fruits. *J. Funct. Foods* 21, 396–405. doi:10.1016/j.jff.2015.12.009

Zeraik, M. L., Ximenes, V. F., Regasini, L. O., Dutra, L. A., Silva, D. H. S., Fonseca, L. M., et al. (2012). 4'-Aminochalcones as novel inhibitors of the chlorinating activity of myeloperoxidase. *Curr. Med. Chem.* 19, 5405–5413. doi:10.2174/092986712803833344

Zhang, D., Tao, X., Gu, G., Wang, Y., Zhao, W., Zhao, W., et al. (2021). Microbial Transformation of neo-Clerodane Diterpenoid, Scutebarbatine F, by Streptomyces sp. CPCC 205437. *Front. Microbiol.* 12, 662321. doi:10.3389/fmicb.2021.662321

Zhang, H., and Chen, S. (2022). Cyclic peptide drugs approved in the last two decades (2001-2021). *RSC Chem Biol* 3, 18–31. doi:10.1039/d1cb00154j