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Brazilian native bamboos: phytochemical study and screening of bioactive molecules with effect on cognition and memory.

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências Biológicas, na Área de Botânica.

Orientadora: Profª Dr.ª Cláudia Maria Furlan

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Orientadora

Ao meu querido filho, com todo meu amor!

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Sumary

Introduction

1. What is known about the medicinal potential of bamboo?

Bamboo is considered one of China's four noble plants, along with orchid, plum, and chrysanthemum. It has inspired the arts like calligraphy, painting, and poetry (Recht and Wetterwald 1992). With the exception of rice, no other plant has played such an important role in the history of China and the East as bamboos, they are present in the daily lives of these populations, from household utensils, food, housing construction, manufacturing paper, and many other uses (Laws 2013).

Bamboo is a grass, belonging to Poaceae, subfamily Bambusoideae, and comprises 1,670 species into 125 genera. The subfamily is divided into 3 tribes: Arundinarieae, Bambuseae, and Olyreae (Soreng et al. 2017). Most species of Arundinarieae (temperate woody bamboos) are almost exclusively from Eurasia, only 3 species can be found in North America; Olyreae (herbaceous bamboos) occurs mainly in the tropical forests of South and Central America; and Bambuseae (woody subtropical and tropical bamboos) has the highest abundance of bamboo species and is distributed throughout the tropical region (Soreng et al. 2017). Therefore, the center of diversity of bamboo species is the Asian continent, followed by South America and the African continent (Das et al. 2008).

Like other plants, bamboos have been used for medicinal purposes for centuries and are described in different Asian pharmacopeias of traditional medicine (Tripathi 2011). For example, in traditional Indian, Chinese, and Tibetan medicine, bamboos are used for the treatment of respiratory diseases, gastrointestinal problems, and for diseases of mental disorders attributed to human aging (Laws 2013; Tripathi 2011).

Sharma and Borthakur (2008) gathered information on bamboo ethnobotanical uses among the Adi tribes of Arunachal Pradesh, India. The residents reported the use of *Bambusa tulda* Roxb for the treatment of tetanus, *Dendrocalamus giganteus* Wall ex. Munro for production of steroid drugs, the decoction of leaves of *Dendrocalamus strictus* (Roxb.) Ness. as abortifacient, and the infusion of leaves of *Schizostachyum capitatum* (Trin.) Rupr. for the treatment of stomach pains.

The alkaline leaves extract from *Sasa senanensis* (Franch. & Sav.) Rehder has been used in Eastern Asia as a potential source of natural drug since hundreds of years ago, and is popularly known as “Sasa health”. Besides that, the leaves extract of this species is used in traditional Japanese medicine as anti-inflammatory (Sangeetha et al. 2015).

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In Xishuangbanna south-eastern of China, people reported that Dai medicine, a local traditional medicine, uses the stem of some bamboo species, as *Dendrocalamus hamilttonii* Ness & Am. Ex Munro, *Thyrsostachys siamensis* Gamble, *Pseudostachyum polymorphum* Munro and *Dendrocalamus* spp., which was an invigorant medicine for kidney (Yang et al. 2008). In a study of the traditional uses of bamboo in the province of Yunnan, in the Chinese Himalayan region, people reported the use of *Phyllostachys glauca* McClure leaves to treat cough and lung inflammation, sap and young culms of *Phyllostachys heterocycla* cv. *pubescens* (Carrière) Matsum. for cough and throat inflammation, and the use of *Indosasa pingbianensis* McClure shoots to treat common cold and headache (Yang et al. 2004).

Kani tribes in India belive that the seeds of *Bambusa arundinacea* (Retz.) Wild. enhance the human fertility, futhermore, the *Bambusa* leaf juice was used to strengthen the cartilage in osteoarthritis and osteoporosis (Sangeetha et al. 2015).

Therefore, the high diversity of species, wide geographic distribution, and the different traditional medicinal uses reported for bamboo raised the question of what we actually know about the chemistry and the biological activities of this group of plants.

This paper compiled studies of the last 11 years about phytochemical and biological activities of bamboo species aiming to address the following questions: which and how many bamboo species have been chemically studied? The Asian continent has the highest diversity of bamboo species, so only Asian species have been studied? What trials have been done to test bamboo medicinal properties?

This review gathered available information on phytochemistry and bioactivity studies of bamboo species from the period of 2007 to 2018. A literature search was performed on Bambusoideae based on major scientific databases including SciELO, SciFinder, Pubmed, and Web of Science. The keywords used were the combination between Bamboo and: biological activities; chemical constituents; phenolic compounds; pharmacological use/activity; traditional uses; flavonoid; potential; chemical characterization; extract; and activity. All scientific names have been checked in the "Taxonomic Name Resolution" website (http://tnrs.iplantcollaborative.org/index.html). Furthermore, only the studies using secondary metabolites characterization were considered for this study, except for some studies that reported biological activities of some primary metabolites. As well as, it was not included in this review studies about sub-products of bamboo, like salt, charcoal, AOB, vinegar, and others.

The aim of this study was to provide perspectives and directions for future research using bamboos as a potential source of drug leads and pharmaceutical agents.

2. Which and how many bamboo species have been studied in the last 11 years?

It was compiled 136 papers regarding phytochemical composition and biological activities of bamboo species. There was an increase on published papers from 2013 to 2018 (Figure 1). More than half of these studies investigated both the phytochemical composition and the biological activity of bamboo species (Figure 2) while 29% of them investigated only the biological activities of bamboo extracts.

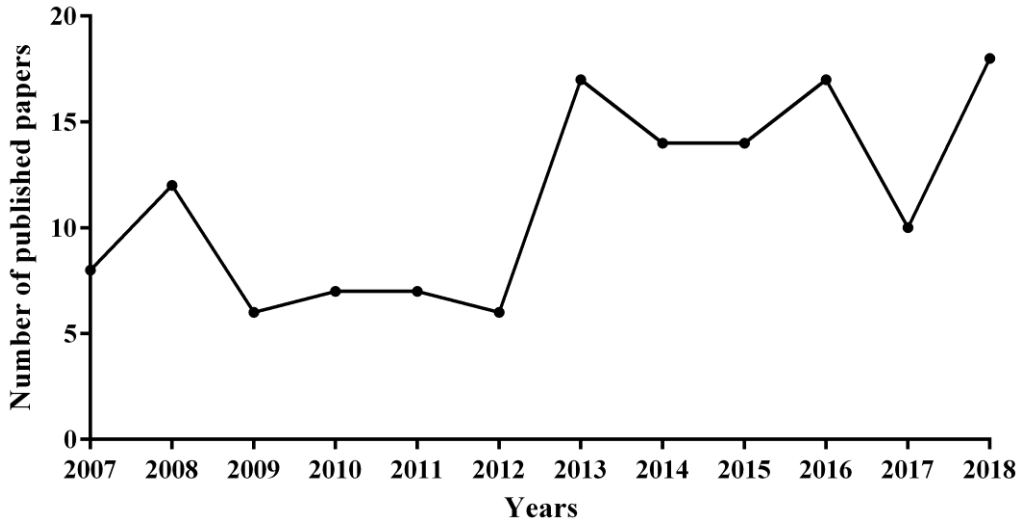


Figure 1 – Total published papers from 2007 to 2018 about phytochemistry and biological activities from bamboo species.

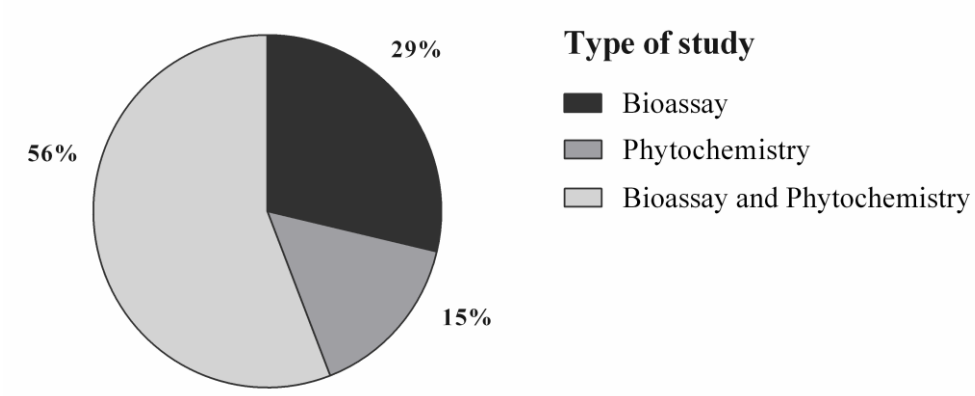


Figure 2 – Percentage of total published papers from 2007 to 2018 divided according to the type of study.

Only 87 bamboo species have been studied and reported in the literature (Table 1), representing 24 genera (Figure 3). It means that 19% of Bambusoideae genera have been studied regarding bioactivity and chemical composition. However, only 5% of bamboo species was accessed, which makes this group of plants with great unexplored potential.

Table 1 –Bamboo species with phytochemical and/or biological study reported from 2007 to 2018.

Accepted name (= Synonym used)	Reference
Tribe Arundinarieae	
Arundinaria gigantea (Walter) Muhl.	Van Hoyweghen et al. 2012
Chimonobambusa quadrangularis (Fenzl) Makino	Chen et al. 2018; Zhang et al. 2018
Fargesia denudata T.P.Yi	Keski-Saari et al. 2008
Fargesia robusta T.P.Yi	Van Hoyweghen et al. 2010; Keski-Saari et al. 2008
Fargesia robusta "Pingwu" T.P.Yi	Van Hoyweghen et al. 2012
Fargesia rufa T.P.Yi	Keski-Saari et al. 2008
Fargesia rufa"Green panda" T.P.Yi	Van Hoyweghen et al. 2012
Fargesia scabrida T.P.Yi	Keski-Saari et al. 2008
Indocalamus latifolius (Keng) McClure	Jia Sun et al. 2015 (b); Ni et al. 2013 (a); Jia Sun et al. 2016
Phyllostachys aurea Rivière & C.Rivière	Racovita and Jetter 2016
Phyllostachys aureosulcata McClure (*Phyllostachys spectabilis*)	Neményi et al. 2015; Wang et al. 2013
Phyllostachys aureosulcata f. *aureocaulis* Z.P.Wang & N.X.Ma	Neményi et al. 2015
Phyllostachys aureosulcata f. *spectabilis* (Chu & Chao) C.D.Chu & C.S.C	Neményi et al. 2015
Phyllostachys bambusoides Siebold & Zucc.	Kumar et al. 2014; Li et al. 2008; Lee et al. 2008; Hong et al. 2010; Kwon et al. 2017; Zhao et al. 2017
Phyllostachys bissetii McClure	Neményi et al. 2015; Kweon et al. 2007; Lin et al. 2008; Wedler et al. 2014; Yang et al. 2014
Phyllostachys edulis (Carrière) J.Houz. (*Phyllostachys pubescens*)	Panee et al. 2008; Chou et al. 2014; Pang and Panee, 2016; Tanaka et al. 2011; Hong et al. 2010; Afrin et al. 2012; Xie et al. 2013; Tanaka et al. 2014; Tanaka et al. 2013; Sun et al. 2017; Park and Jhon 2010; Choi et al. 2013; Zhu et al. 2018
Phyllostachys flexuosa Rivière & C.Rivière	Neményi et al. 2015
Phyllostachys glauca McClure	Guo et al. 2013
Phyllostachys heterocycla (Carrière) S. Matsum.	Yoshimura et al. 2017; Liu et al. 2018
Phyllostachys heterocycla cv. *Pubescens* (Carrière) S. Matsum.	Ming et al. 2015; Duan et al. 2017; Tao et al. 2017
Phyllostachys humilis	Van Hoyweghen et al. 2012; Neményi et al. 2015
Phyllostachys iridescens C.Y.Yao & S.Y.Chen	Neményi et al. 2015
Phyllostachys mannii Gamble	Neményi et al. 2015
Phyllostachys nidularia Munro	Wang et al. 2013
Phyllostachys nigra (Lodd. ex Lindl.) Munro	Van Hoyweghen et al. 2012; Van Hoyweghen et al. 2014; Shang et al. 2014; Shin et al. 2016; Park and Jhon, 2010; Kim et al. 2009; Jung et al. 2007; Shang et al. 2016; Park and Jhon 2009; Hong et al. 2010; Wang et al. 2013
Phyllostachys nigra var. *henonis* (Mitford) Rendle	Kim et al. 2007; Zhang et al. 2010; Jiao et al. 2007 (a); Gong et al. 2015; Neményi et al. 2015; Choi et al. 2018; Jiao et al. 2007 (b); Zhang et al. 2008; Wang et al. 2010
Phyllostachys nigra var. *nigra* (Lodd. ex Lindl.) Munro	Neményi et al. 2015
Phyllostachys parvifolia C.D.Chu & H.Y.Chou	Patel et al. 2016

Accepted name (= Synonym used)	Reference
Phyllostachys propinqua McClure	Wang et al. 2013
Phyllostachys sulphurea (Carrière) Rivière & C.Rivière	Wang et al. 2013
Phyllostachys sulphurea var. *sulphurea* (Carrière) Rivière & C.Rivière	Neményi et al. 2015
Phyllostachys violascens Rivière & C.Rivière	Neményi et al. 2015
Phyllostachys viridiglaucescens (Carrière) Rivière & C.Rivière	Neményi et al. 2015
Phyllostachys vivax f. *aureocaulis* N.X.Ma	Neményi et al. 2015
Pleioblastus argenteostriatus (Regel) Nakai (*Sasa argenteostriata*)	Ni et al. 2012; Wang et al. 2013
Pleioblastus fortunei (Van Houtte) Nakai (*Pleioblastus variegatus*)	Wang et al. 2013; Van Hoyweghen et al. 2012
Pleioblastus kongosanensis f. *aureostriatus* Muroi & Yu.Tanaka	Ni et al. 2013 (b); Ni et al. 2013(c)
Pseudosasa japonica (Steud.) Makino	Van Hoyweghen et al. 2012; Wang et al. 2013
Sasa borealis (Hack.) Makino & Shibata	Oh et al. 2013; Park et al. 2007; Choi et al. 2008
Sasa kurilensis (Rupr.) Makino & Shibata (*Sasa coreana*)	Yang et al. 2017
Sasa kurilensis var. *gigantea* Tatew.	Hasegawa et al. 2008
Sasa palmata (Burb.) E.G.Camus	Kurosumi et al. 2007; Zulkafli et al. 2014
Sasa quelpaertensis Nakai	Kang and Lee 2015; Kim et al. 2014; Hwang et al. 2007; Ko et al. 2018; Herath et al. 2018 (a); An et al. 2008; Sultana and Lee 2009; Sultana and Lee 2010; Herath et al. 2018 (b)
Sasa senanensis (Franch. & Sav.) Rehder	Seki et al. 2008; Khatun et al. 2013; Matsuta et al. 2009; Sakagami et al. 2018; Matsuta et al. 2011
Sasa veitchii (Carrière) Rehder (*Sasa albo-marginata*)	Yoshioka et al.2017; Yoshioka et al. 2016; Sato et al. 2016; Shirotake et al. 2009; Van Hoyweghen et al. 2014; Sato et al. 2015; Van Hoyweghen et al. 2012; Sakai et al. 2008; Akuzawa et al. 2011
Shibataea chinensis Nakai	Ni et al. 2013 (b)
Yushania brevipaniculata (Hand.-Mazz.) T.P.Yi (*Yushania chungii*)	Keski-Saari et al. 2008
Tribe Bambuseae	
Aulonemia aristulata (Döll) McClure	Grombone-Guaratini et al. 2009
Bambusa arundinaceae (Retz.) Willd.	Zubair et al. 2013; Vanitha et al. 2016; Manohari et al. 2016; Abirame et al. 2018; Joselin et al. 2014
Bambusa balcooa Roxb.	Goyal et al. 2017; Van Hoyweghen et al. 2012
Bambusa bambos (L.) Voss (*Bambusa bambose*)	Sriraman et al. 2015; Wasnik and Tumane 2014; Soumya et al. 2016
Bambusa emeiensis L.C.Chia & H.L.Fung (*Neosinocalamus affinis; Sinocalamus affinis*)	Luo et al. 2015; Jia Sun et al. 2013 (b); Xia Hu et al. 2018
Bambusa heterostachya (Munro) Holttum	Joselin et al. 2014
Bambusa nutans Wall. ex Munro	Tripathi et al. 2015; Pande et al. 2018
Bambusa pervariabilis McClure	Jia Sun et al. 2010; Wang et al. 2013
Bambusa polymorpha Munro	Thomas et al. 2016
Bambusa rutila McClure	Gao et al. 2012
Bambusa textilis McClure	Silva et al. 2012; Liu et al. 2016
Bambusa tulda Roxb.	Pande et al. 2017; Lee et al. 2018

Accepted name (= Synonym used)	Reference
Bambusa tuldoides Munro	Jia Sun et al. 2013 (a); Jia Sun et al. 2015 (a)
Bambusa ventricosa McClure	Coffie et al. 2014; Joselin et al. 2014
Bambusa vulgaris Schrad. (*Bambusa madagascariensis*)	Owolabi and Lajide 2015; Senthilkumar et al. 2011; Tripathi et al. 2015; Joselin et al. 2014; Ballhorn et al. 2016
Bambusa vulgaris var. *vittata* Schrad.	Goyal et al. 2013; Coffie et al. 2014
Bambusa vulgaris var. *vulgaris*	Coffie et al. 2014
Cathariostachys capitata (Kunth) S.Dransf.	Ballhorn et al. 2016
Cathariostachys madagascariensis (A.Camus) S.Dransf.	Ballhorn et al. 2016
Cephalostachyum sp.	Ballhorn et al. 2016
Dendrocalamopsis oldhami (Munro) Keng F.	Zhao-Lin et al. 2012
Dendrocalamus asper (Schult.) Backer	Jingli Zhang et al. 2018
Dendrocalamus giganteus Munro	Wang et al. 2013
Dendrocalamus hamiltonii Nees & Arn. ex Munro	Van Hoyweghen et al. 2012; Li et al. 2018
Dendrocalamus latiflorus Munro	Li et al. 2018; Zheng et al. 2014; Chang et al. 2013
Dendrocalamus strictus (Roxb.) Nees	Goyal et al. 2011; Joselin et al. 2014
Dinochloa scandens (Blume ex Nees) Kuntze	Van Hoyweghen et al. 2012
Gigantochloa levis (Blanco) Merr.	Tongco et al. 2016
Gigantochloa ligulata Gamble	Ilham et al. 2008
Gigantochloa scortechinii Gamble	Ilham et al. 2008
Guadua amplexifólia J.Presl	Van Hoyweghen et al. 2012
Guadua angustifolia Kunth	Mosquera et al. 2015; Álvarez et al. 2015; Valencia et al. 2011; Martínez et al. 2015
Melocanna baccifera (Roxb.) Kurz	Khan et al. 2018; Govindan et al. 2016; Govindan et al. 2018
Merostachys pluriflora Munro ex E.G.Camus	Faria and Grombone-Guaratini, 2011; Gagliano et al. 2018
Merostachys riedeliana Rupr. ex Döll	Jose et al. 2016
Merostachys skvortzovii Send.	Sanquetta et al. 2013
Nastus elongatus A.Camus	Ballhorn et al. 2016
Oxytenanthera abyssinica (A.Rich.) Munro	Bartholomew et al. 2013; Coffie et al. 2014
Schizostachyum lumampao (Blanco) Merr.	Tongco et al. 2014
Schizostachyum zollingeri Steud.	Ilham et al. 2008

The most studied genera of this subfamily were *Phyllostachys* (25 species), followed by *Bambusa* (16 species) and *Sasa* (7 species) (Figure 3 and 4; Table 1). *Phyllostachys* and *Sasa* belong to Arundinarieae tribe which contains 581 species and 31 genera (Soreng et al. 2017). For this tribe, 32% of the genera were studied. *Bambusa* belongs to Bambuseae tribe, which includes 966 species and 73 genera (Soreng et al. 2017), being 19% of these genera already studied.

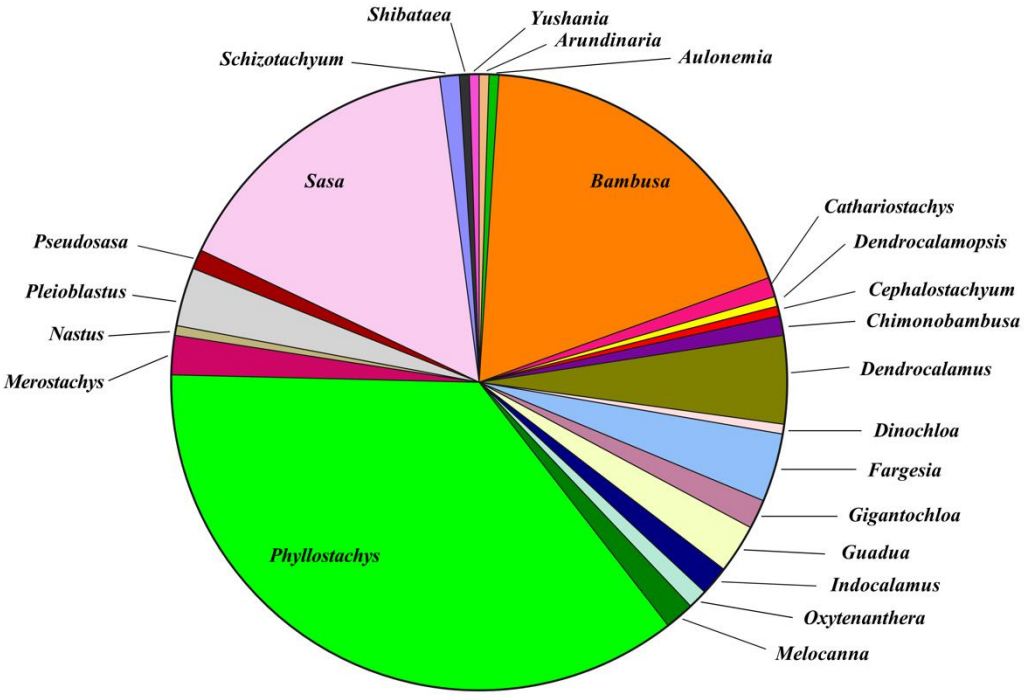


Figure 3 – Compiled papers from 2007 to 2018 regarding phytochemistry and bioactivity studies of bamboo genera.

Bambusoideae are divided into two morphologically distinct habits: woody and herbaceous bamboos. While woody bamboos display a wide range of morphological diversity, they do possess multiple shared characteristics, as strongly lignified culms, specialized culm leaves, complex vegetative branching, outer ligules on the foliage leaves, bisexual flowers, and gregarious monocarpy. Furthermore, some of these bamboos which can quickly grow up to 45 m in height, serve as an economically important source of building materials and other products for cultures in Asia, Africa, Australia, and Central and South Americas (Wysocki et al. 2015).

China has the most abundant bamboo resources worldwide and the richest bamboo uses. The national bamboo forest covers 6.01 million ha, including 4.43 million ha of Moso bamboo (*Phyllostachys edulis* (Carrière) J. Houz.) and 1.58 million ha of other bamboo species (Weiyi et al. 2018).

P. edulis is a major economic species grown in subtropical regions, which constitutes the largest artificial bamboo formation and has been thoroughly exploited. Futhermore, this species is largely spread due to its cultivation (Weiyi et al. 2018).

Figure 4 –Some of the most studied bamboo species from Arundinarieae and Bambuseae. Photos source: asianflora.com (accessed in Oct/2020).

According to Yeasmin et al. (2015), India is the second richest country in bamboo genetic resources following China. Around 13% of the total forest area, which is about 9.57 million ha of this country, is covered by bamboo plantation. *Dendrocalamus* and *Bambusa* are the two most predominant bamboo species distributed in subtropical, tropical moist and tropical dry agroclimatic zones of India. Therefore, the wide distribution and cultivation of *Phyllostachys* spp. in China and *Bambusa* spp. in India can explain the greater number of studies with these genera compiled in this review.

It was not found any phytochemical study using species from the Olyreae tribe (herbaceous bamboo). This tribe includes 123 species and 21 genera, occurring primarily in the tropical forests of South and Central America, exception for *Buergersiochloa* Pilg. found in Malaysia and *Olyra latifolia* L. that occurs in Africa (Soreng et al. 2017).

These data show a gap of information regarding bamboo species from the New World. In the Americas, Brazil concentrates the greatest diversity of herbaceous bamboo, being reported the occurrence of 16 genera; four of them are endemic (*Diandrolyra* Stapf, *Eremitis* Döll, *Reitzia* Swallen, and *Sucrea* Soderstr.). *Olyra* L. and *Pariana* Aubl., comprising 18 species each, are the largest genera in number of species (Filgueiras and Gonçalves 2004). Furthermore, in Brazil, it was reported the occurrence of 75 species of herbaceous bamboos, being 45 (60%) of them endemic. Therefore, there is an entire group of bamboo species chemically unexplored.

Herbaceous bamboos are characterized by shorter and more weakly lignified shoots, less vegetative branching, unisexual flowers, and annual or seasonal flowering patterns; they have a much more restricted geographic distribution (Wysocki et al. 2015).

Bamboos have a great economic and cultural importance for several Asian countries. Herbaceous bamboos have an almost exclusive distribution in the New World, which may explain the inexistence of studies with economic bias with these bamboo species. Furhtermore, morphological characteristics can favor the use of woody bamboo species in different segments of the industry, which may explain the great use of these species in detriment to the herbaceous and consequently the greater number of studies with woody bamboo species, as noted in this review.

Leaves were the most studied organ, followed by shoots and culms, for phytochemical and bioactivity studies (Figure 5). Perhaps a large number of studies using leaves can be attributed to the fact that this organ is considered a waste, since the culms of many Asian species are used in construction and for furniture-making industry.

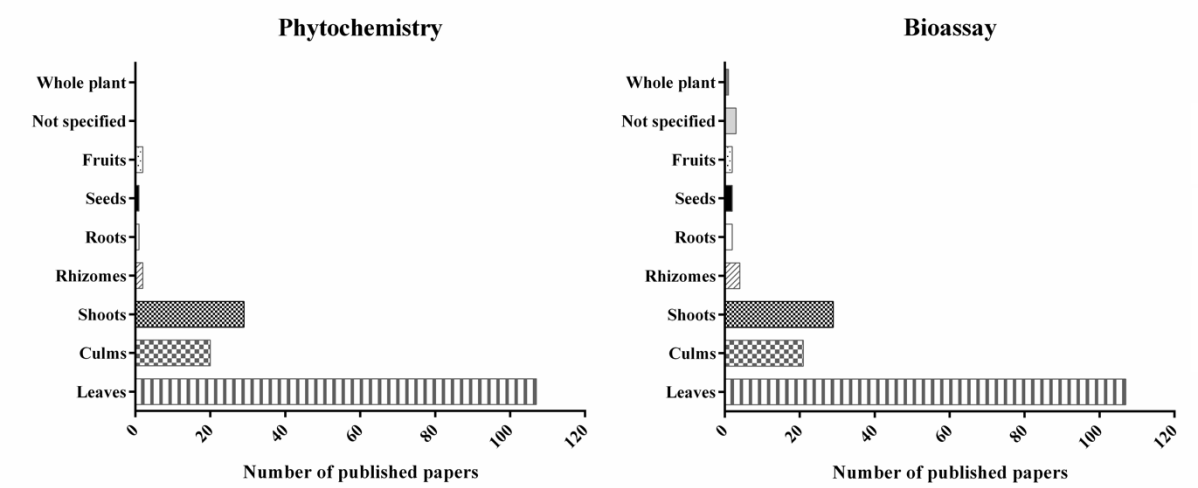


Figure 5 – Percentage of papers reporting chemical composition and biological activities for different plant organs.

3. Bamboo chemical profile

The major class of secondary metabolites reported for bamboo species is the phenolic compounds (Figure 6). From 136 papers, 77 (56%) of them reported the phenolic composition of bamboo species (Table 2). Flavones, as apigenin and luteolin derivatives, and hydroxycinnamic acid derivatives were detected in almost all the studied species. The most common flavones were *C*-glycosides, *e.g*. vitexin, isovitexin, and orientin (Van Hoyweghen et al. 2012; Pande et al. 2018; Lv et al. 2012), while the most frequent hydroxycinnamic acid derivatives reported were caffeic, ferulic, *p*-coumaric (Pande et al. 2017), and chlorogenic acids (Ni et al. 2013a).

Coumarin is another well-reported class of phenolic compounds in bamboos. Wang et al. (2013) developed a simple, rapid, and sensitive HPLC-UV method for qualitative and quantitative analysis of foliar coumarins in 11 bamboo species, detecting skimin, scopolin, scopoletin, umbelliferone, 6,7-dimethoxycoumarin, coumarin, psoralen, xanthotoxin, 5,7-dimethoxycoumarin, pimpinellin, imperatorin, and osthole.

Phenolics are defined as compounds that possess an aromatic ring with at least one hydroxyl group, and their structure can vary from simple molecule to complex polymer with high molecular weight. The most adopted classification implies the subdivision of phenolics in two main groups: flavonoids and non-flavonoid polyphenols, and this classification have been commonly used in the literature (Durazzo et al. 2019).

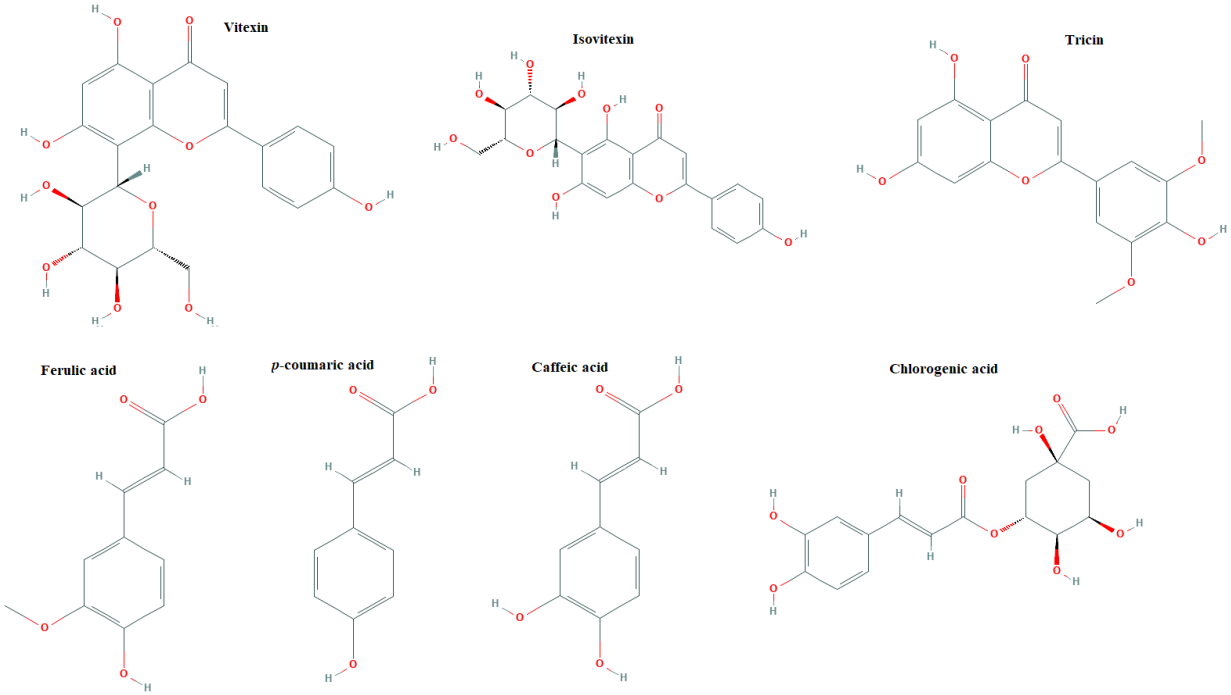


Figure 6 –Some most commom secondary metabolites reported in bamboo species. Figure source: Pubchem, 2020.

Phenolic compounds are ubiquitous in plants and play important ecological functions for them, as photoprotection, mechanical support, and attractive for pollinators and fruit dispersers (Dewick 2009). For humans, phenolic substances have an increasing interest for health applications due to a wide range of their biological activities, in especial the antioxidant activity towards cancer, cardiovascular, and neurodegenerative diseases, or for uses in antiaging products (Boudet 2007).

Polyphenols are also the major active compounds present in teas, the most widely used beverage worldwide. Asian people have been drinking tea for centuries, and it is known that the benefits to human health are attributed to polyphenols present in teas (Khan and Mukhtar 2019). Perhaps the different traditional uses of bamboo are directly associated with the polyphenols found in these species.

Although there are studies reporting the presence of alkaloids, these were detected using preliminary screening methods (Table 2), exception for 2 studies that isolated and identified serotonin alkaloids in two bamboo species using HPLC and NMR. *N*-*p*-coumaroylserotonin and *N*-feruloylserotonin were reported in leaves of *Sasa quelpaertensis* Nakai (Sultana and Lee 2009); and *N*-feruloylserotonin was also identified in leaves of *Phyllostachys nigra* (Lodd. ex Lindl.) Munro (Shang et al. 2014).

Only 27 papers reported the use of NMR for structure elucidation, most of the studies used HPLC and co-chromatography with authentic compounds, or applied colorimetric methods (Figure 7). These data show that only few studies have been able to isolate and characterize bamboo constituents.

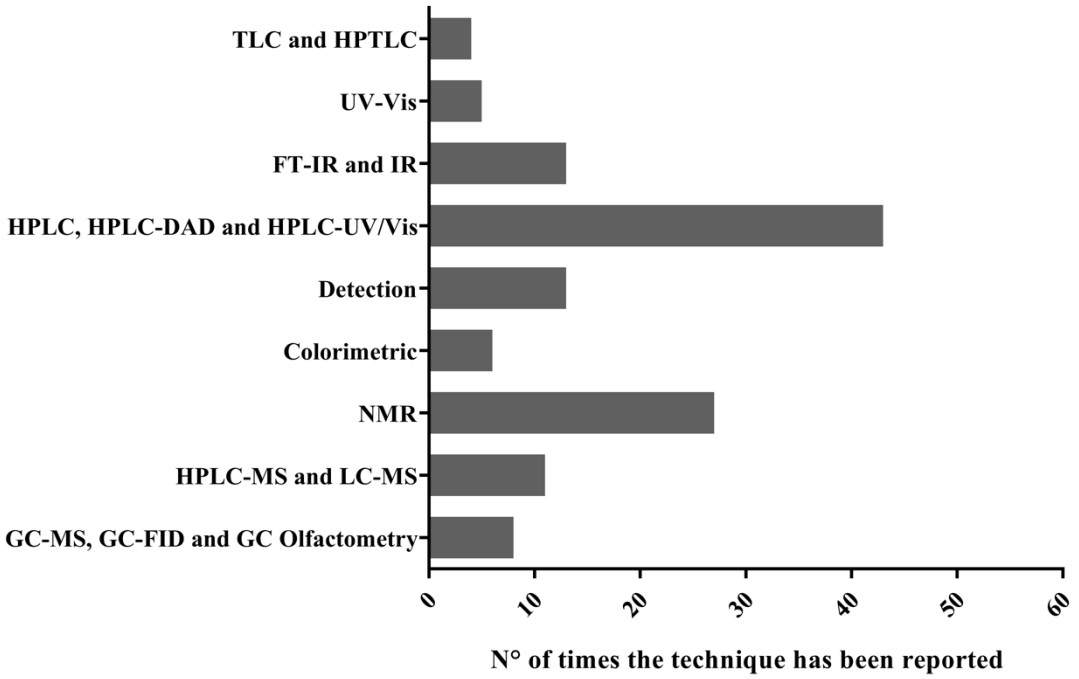


Figure 7 – Number of times that the identification techniques have been reported.

Table 2 –Phytochemical studies reported from 2007 to 2018.

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Arundinaria gigantea	Leaves	HPLC-DAD and LC-MS/MS	*C*-hexosyl-*C*-pentosyl-apigenin, Di-*C*,*C*-pentosyl-apigenin, Di-*O*,*C*-pentosyl-apigenin, *C*-hexosyl-*O*-pentosyl-luteolin, *O*-hexosyl-C-hexosyl luteolin, isoorientin, *O*-hexosyl-*O*-deoxyhexosyl-tricin, *O*-hexosyl-tricin, dihydroxybenzoic acid hexose, coumaroyl-quinic acid, caffeoyl quinic acid, coumaroyl hexose, sinapoyl hexose, and sinapic acid derivative.	Van Hoyweghen et al. 2012
Aulonemia aristulata	Leaves	HPLC-UV with authentic standards	Quercetin, rutin, and ferulic acid.	Grombone-Guaratini et al. 2009
Bambusa arundinaceae	Shoots	Detection methods	Phenolic compounds, glycosides, steroids, tannins, carbohydrates, and proteins.	Vanitha et al. 2016
Bambusa arundinaceae	Leaves	FT-IR and Detection methods	Alkaloids, phenolic compounds, saponins, glycosides, terpenoids, steroids, phytosterols, and coumarins.	Joselin et al. 2014
Bambusa arundinaceae	Seeds	Detection methods	Tannins, phlobatannins, flavonoids, cardiac glycosides, and reducing sugars.	Manohari et al. 2016
Bambusa arundinaceae	Leaves	GC-MS	*n*-nonane, trimethylbutane, tridecane, and 3,4,5,6-tetramethyloctane.	Zubair et al. 2013
Bambusa balcooa	Leaves	HPLC-UV and colorimetric methods	Rutin, gallic acid, and *β*-sitosterol.	Goyal et al. 2017
Bambusa balcooa	Leaves	HPLC-DAD and LC-MS/MS	*C*-hexosyl-*C*-pentosyl-apigenin, isovitexin, tricin, 5-feruloylquinic acid, and sinapoyl hexose.	Van Hoyweghen et al. 2012
Bambusa bambos	Leaves	HPTLC and RP-HPLC-PDA	β-sitosterol and stigmasterol.	Sriraman et al. 2015
Bambusa bambos (= Bambusa bambose)	Leaves	Detection methods	Flavonoids, phenolic acids, tannins, terpenes, saponins, carbohydrates, and proteins.	Wasnik and Tumane 2014

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Bambusa emeiensis (= Neosinocalamus affinis)	Leaves	UV, MS and NMR	Tricin-6-*C*-β-boivinopyranosyl-8-*C*-β-glucopyranoside, 5,7,4'-trihydroxy-3'-methoxy-6-*C*[β-D-xylopyranosyl-(1-2)]-β-D-glucopyranosyl flavonoside, apigenin-6-*C*-β-boivinopyranosyl-7-*O*-β glucopyranoside, luteolin-6-*C*- β-boivinopyranosyl-7-*O*-β-glucopyranoside, isovitexin-2"-xyloside, isoorientin-2"-xyloside, tricin-7-*O*-β-glucopyranoside, isoorientin, and tricin.	Jia Sun et al. 2013 (b)
Bambusa emeiensis (=Neosinocalamus affinis)	Leaves	UV, IR, MS and NMR	4'-*O*-((7"R,8"S)-8"-guaiacylglyceryl)-pleioside B, apigenin 6-*C*-β- D-fucopyranosyl-7-*O*-β-D-glucopyranoside, pleioside A and B, tricin 7-*O*-β-D-glucopyranoside, farobin A, liriodendrin, tricin-7-*O*-neohesperidoside, isoorientin, and (threo)-3-hydroxy-1- (4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(E)-propenyl)-2,6-dimethoxy- phenoxy]-propyl-β-D-glucopyranoside.	Luo et al. 2015
Bambusa heterostachya	Leaves	FT-IR and Detection methods	Alkaloids, phenolic compounds, saponins, glycosides, terpenoids, steroids, phytosterols, and coumarins.	Joselin et al. 2014
Bambusa nutans	Leaves	UV-Vis and Detection methods	Alkaloids, terpenoids, phenolic compounds, tannins, and glycosides.	Tripathi et al. 2015
Bambusa nutans	Leaves	HPLC-ESI-QTOF-MS	Caffeic, *p*-coumaric, sinapic, ferulic, coumaroylquinic, 5-feruloylquinic, and dihydroxybenzoic acids; orientin, tricin, vitexin, isoorientin, isovitexin, luteolin, apigenin, quercitrin, and rutin.	Pande et al. 2018
Bambusa pervariabilis	Leaves	HPLC-DAD, IR, HR-ESI-MS and NMR	7,8-dihydroxy-3-(3-hydroxy-4-oxo-4H-pyran-2-yl)-2H-chromen-2-one, scopoletin, and scopolin.	Jia Sun et al. 2010
Bambusa pervariabilis	Leaves	HPLC-UV	Scopolin and scopoletin.	Wang et al. 2013
Bambusa rutila	Leaves	UV-Vis	Four kinds of polysaccharides.	Gao et al. 2012
Bambusa textilis	Leaves	Detection methods	Alkaloids, tannins, phenolic compounds, and saponins.	Silva et al. 2012

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Bambusa textilis	Leaves	UFLC-Q-TOF-MS	Gallic acid, 3,4-dimethoxyphenyl β-D-glucopyranoside, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *O*-hexosyl-*C*-hexolyl luteolin or isomer, *p*-coumaric acid, 7-*O*-glucosyl-6-*C*-glucosyl apigenin, 4''-*O*-xylosyl-isoorientin, 8-*C*-xylosyl-6-*C*-glucosyl apigenin, orientin, 2''-*O*-rhamnosyl isoorientin, isoorientin, vitexin, 2''-*O*-xylosyl isovitexin, 2''-*O*-rhamnosyl isovitexin, isovitexin, 7-*O*-glucosyl luteolin, 4'-*O*-glucosyl-6-*C*-digitoxosyl luteolin, 6-*C*-arabinosyl luteolin, 6-*C*-boivinosyl-7-*O*-glucosyl apigenin, 2''-*O*-apiosyl-7-*O*-glucosyl tricin, 4'-*O*-glucosyl tricin, 7-*O*-glucosyl tricin, 4'-*O*-guaiacylglyceryl-7-*O*-glucosyl tricin, luteolin, tricin, apigenin; α-linolenic, linoleic, palmitic, oleic, and stearic acids.	Liu et al. 2016
Bambusa tulda	Leaves	HPLC-ESI-QTOF-MS	Caffeic, coumaroylquinic, dihydroxybenzoic, 5-feruloylquinic, sinapic, ferulic, and *p*-coumaric acids; orientin, vitexin, quercetrin, isoorientin, isovitexin, and tricin.	Pande et al. 2017
Bambusa tuldoides	Culms	HPLC-DAD, LC-MS and NMR	(−)-7′-epi-lyoniresinol 4,9′-di-*O*-β-D-glucopyranoside, (−)-lyoniresinol 4,9′-di-*O*-β-D-glucopyranoside, bambulignan A, (+)-lyoniresinol 9′-*O*-β-D-glucopyranoside, 18−20 (−)-lyoniresinol 9′-*O*-β-D-glucopyranoside, 21 (−)-5′-methoxyisolariciresinol 9′-*O*-β-glucopyranoside, 22 (+)-lyoniresinol, 23 (+)-lyo-niresinol 4-*O*-β-D-glucopyranoside, 24 (−)-lyoniresinol 9-*O*- β-D-glucopyranoside, and 25 (−)-7′-epilyoniresinol 9′-*O*-β- D-glucopyranoside.	Jia Sun et al. 2013 (a)
Bambusa tuldoides	Leaves	HPLC-UV, CD and NMR	Oxyneolignans A, B, C, and D.	Jia Sun et al. 2015 (a)
Bambusa ventricosa	Leaves	Detection methods	Flavonoids, saponins, general glycosides, coumarins, and cyanogenic glycosides.	Coffie et al. 2014
Bambusa ventricosa	Leaves	FT-IR and Detection methods	Alkaloids, phenolic compounds, saponins, glycosides, terpenoids, steroids, phytosterols, and coumarins.	Joselin et al. 2014
Bambusa vulgaris	Leaves	FT-IR and Detection methods	Alkaloids, phenolic compounds, saponins, glycosides, terpenoids, steroids, phytosterols, and coumarins.	Joselin et al. 2014
Bambusa vulgaris	Leaves	Detection methods	Phenolic compounds, flavonoids, terpenoids, alkaloids, and tannins.	Owolabi and Lajide 2015
Bambusa vulgaris	Leaves	Detection methods	Phytosterols and tannins.	Senthilkumar et al. 2011

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Bambusa vulgaris	Leaves	UV-Vis and Detection methods	Alkaloids, terpenoids, phenolic compounds, tannins, and glycosides.	Tripathi et al. 2015
Bambusa vulgaris (= Bambusa madagascariensis)	Shoots	Colorimetric methods	It was found variation in cyanogenic potential (HCNp).	Ballhorn et al. 2016
Bambusa vulgaris var. *vittata*	Leaves	Detection methods	Saponins, general glycosides, coumarins, and cyanogenic glycosides.	Coffie et al. 2014
Bambusa vulgaris var. *vulgaris*	Leaves	Detection methods	Saponins, general glycosides, coumarins, and cyanogenic glycosides.	Coffie et al. 2014
Cathariostachys capitata	Shoots	Colorimetric methods	It was found variation in cyanogenic potential (HCNp).	Ballhorn et al. 2016
Cathariostachys madagascariensis	Shoots	Colorimetric methods	It was found variation in cyanogenic potential (HCNp).	Ballhorn et al. 2016
Cephalostachyum sp.	Shoots	Colorimetric methods	It was found variation in cyanogenic potential (HCNp).	Ballhorn et al. 2016
Chimonobambusa quadrangularis	Shoots	HPLC; FT-IR and NMR	Polysaccharides were identified as hetero-polysaccharides, composed of Man, Rha, GlcA, GalA, Glc, Gal, Xyl, and Ara.	Chen et al. 2018
Chimonobambusa quadrangularis	Culms	HPLC and FT-IR	Polysaccharides composed of Man, Rha, GlcA, Glc, Gal, Xyl and Ara.	Zhang et al. 2018
Dendrocalamopsis oldhami	Leaves	HPLC-DAD	Chlorogenic acid, caffeic acid, *C*-Hexosyl *O*-pentosyl luteolin, isoorientin, orientin, *C*-hexosyl *O*-hexosyl aglycones, vitexin, isovitexin, *C*-hexosyl luteolin, *O*-rutinoside apigienin, *O*-rutinoside tricin, *O*-hexosyl tricin, luteolin, and tricin.	Zhao-Lin et al. 2012
Dendrocalamus asper	Shoots	co-TLC, CG-MS and NMR	4-hydroxybenzoic acid and *p*-hydroxybenzaldehyde, ketosteroid, cholest-4-en-3-one, lauric and palmitic acids.	Jingli et al. 2018
Dendrocalamus giganteus	Leaves	HPLC-UV	Scopolin and scopoletin.	Wang et al. 2013
Dendrocalamus hamiltonii	Leaves	HPLC-DAD; LC-MS/MS	*C*-hexosyl-*C*-pentosyl-apigenin, *C*-hexosyl-apigenin, *O*-hexosyl-*O*-deoxyhexosyl-tricin, rutin, and sinapoyl hexose.	Van Hoyweghen et al. 2012
Dendrocalamus latiflorus	Shoots	GC-MS and GC-Olfactometry	Ethanol, 1-hexanol, hexanal, methoxy-phenyl-oxime, acetic acid, 2-pentylfuran, hexanal, benzaldehyde, 1-hexanol, and (E)-2-nonadienal.	Zheng et al. 2014

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Dendrocalamus latiflorus	Shoots	GC-FID	*p*-hydroxybenzaldehyde, vanillin, syringaldehyde; *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, and ferulic acids.	Chang et al. 2013
Dendrocalamus strictus	Leaves	FT-IR and Detection methods	Alkaloids, phenolics, saponins, glycosides, terpenoids, steroids, phytosterols, and coumarins.	Joselin et al. 2014
Dinochloa scandens	Leaves	HPLC-DAD; LC-MS/MS	*C*-hexosyl-*C*-pentosyl-apigenin and sinapoyl hexose.	Van Hoyweghen et al. 2012
Fargesia denudata	Culms	HPLC-DAD, HPLC-MS and HPLC-ESI-MS	*p*-OH-cinnamic acid, vicenin, luteolin derivative, isoorientin rhamnoside, and apigenin derivative.	Keski-Saari et al. 2008
Fargesia robusta	Culms	HPLC-DAD, HPLC-ESI-MS, HPLC-MS	Neochlorogenic acid, methylchlorogenic acid, *p*-OH-cinnamic acid, and chlorogenic acid derivative.	Keski-Saari et al. 2008
Fargesia robusta	Leaves	HPLC-UV, MS and NMR	Farobin A and B, tricin 5-*O*-β-D-glucopyranoside, 2''-*O*-α-rhamnosyl-6-*C*-(6-deoxy-ribo-hexos-3-ulosyl)-luteolin, and isoorientin.	Van Hoyweghen et al. 2010
Fargesia robusta "Pingwu"	Leaves	HPLC-DAD; LC-MS/MS	*C*-glycosyl-chrysoeriol derivative, isoorientin, farobin A and B, cassiaoccidentalin B, *O*,*C*-dideoxyhexosyl-luteolin, *O*-deoxyhexosyl-*C*-deoxyhexosylluteolin, tricin-5-*O*-glucoside, tricin;,dihydroxybenzoic acid hexose, 5-feruloylquinic acid, and sinapic acid derivative.	Van Hoyweghen et al. 2012
Fargesia rufa	Culms	HPLC-DAD, HPLC-MS and HPLC-ESI-MS	Neochlorogenic acid, methylchlorogenic acid derivative, *p*-OH-cinnamic acid, chlorogenic acid derivative, vicenin, luteolin derivative, isoorientin rhamnoside, and apigenin derivative.	Keski-Saari et al. 2008
Fargesia rufa "Green panda"	Leaves	HPLC-DAD; LC-MS/MS	Isoorientin, farobin A, cassiaoccidentalin B, *O*,*C*-dideoxyhexosyl-luteolin, tricin-5-*O*-glucoside, *O*-hexosyl-tricin, and 5-feruloylquinic acid.	Van Hoyweghen et al. 2012
Fargesia scabrida	Culms	HPLC-DAD, HPLC-MS and HPLC-ESI-MS	Chlorogenic acid derivative, vicenin-1, apigenin derivative, and chlorogenic acid derivative.	Keski-Saari et al. 2008
Guadua amplexifolia	Leaves	HPLC-DAD; LC-MS/MS	Di-*C*,*C*-hexosyl-apigenin, *C*-hexosyl-*C*-pentosyl-apigenin, di-*C*,*C*-pentosyl-apigenin, *O*-hexosyl-*O*-deoxyhexosyl-apigenin, di-*C*-glycosyl-apigenin, *C*-glycosyl-apigenin derivate, *C*-glycosyl-chrysoeriol derivative, and sinapoyl hexose.	Van Hoyweghen et al. 2012

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Guadua angustifolia	Culms	TLC and HPLC-DAD	Alkaloids, phenols, flavonoids, terpenes, and steroidal saponins.	Mosquera et al. 2015
Guadua angustifolia	Culms	Detection methods	Flavonoids, alkaloids, terpenoids, and saponins.	Martínez et al. 2015
Guadua angustifolia	Leaves	Detection methods	Cardiac glycosides, aminoacids, terpenoids, steroids, and phenolic compounds.	Álvarez et al. 2015
Indocalamus latifolius	Leaves	Polarimeter, HPLC-DAD, FT-IR, CD, HRESIMS and NMR	Erythro-syringylglycerol-9-*O*-*trans*-4-hydroxycinnamate 7-*O*-β-D-glucopyranoside, indocalatin A; 5,7,3′-trihydroxy-6-C-β-D-digitoxopyranosyl-4′-*O*-β-D-glucopyranosyl flavonoid, 5,4′-dihydroxy-3′,5′-dimethoxy-7-*O*-[β-D-apiose-(1→2)]-β-D-glucopyranosy flavonoid, and tricin-6-C-β-boivinopyranosyl-8-C-β-glucopyranoside.	Jia Sun et al. 2016
Indocalamus latifolius	Leaves	HRESIMS, CD and NMR	Latifoliusine A, (7S,8R) syringylglycerol-8-*O*-4′-sinapyl ether 4-*O*-β-D-glucopyranoside, (7S,8S) syringylglycerol-8-*O*-4′-sinapyl ether 7-*O*-β-D-glucopyranoside, (7R,8S) syringylglycerol-8-*O*-4′-sinapyl ether 7-*O*-β-D-glucopyranoside, L-phenylalanine, dihydroxymethyl-bis(3,5-dimethoxy-4-hydroxyphenyl) tetrahydrofuran-9-*O*-β-D-glucopyranoside, rel-(7R,8S,7′S,8′R)- 4,9,4′,9′-tetrahydroxy-3,3′-dimethoxy-7,7′-epoxylignan 9-*O*-β-D-glucopyranoside, apigenin 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranoside, apigenin 7-*O*,8-*C*-di-glucopyranoside, and (7S,8S) syringylglycerol-8-*O*-4′-sinapyl ether 9′-*O*-β-D-glucopyranoside.	Jia Sun et al. 2015 (b)
Indocalamus latifolius	Leaves	UV and RP-HPLC	Orientin, isoorientin, vitexin, isovitexin; *p*-coumaric, chlorogenic, caffeic, and ferulic acids.	Ni et al. 2013 (a)
Melocanna bacífera	Fruits	HPTLC and Colorimetric methods	Ferulic acid.	Govindan et al. 2016

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Melocanna baccifera	Fruits and leaves	FT-NMR, UPLC-Q-TOF-MS and DART-MS	4-oxabicyclo [3.2.2]nona-1(7),5,8-triene, verbacine, mixture of β-sitosterol and stigmasterol, hexadecanoic acid, mixture of α-amyrenone and β-amyrenone, mixture of β-sitosterol-3-*O*-glucoside and stigmasterol-3-*O*-glucoside, ferulic acid methyl ester, cinnamic acid, hexacosanal, β-sitosterol fatty ester, 3β-friede-linol, 3(20)-phytene-1,2-diol, 5(6)-gluten-3α-ol, E-phytol, mixture of α-amyrin and β-amyrin, 4-hydroxybenzaldehyde, 7,3′,5′-tri-*O*-methyltricetin, syringic acid, blumenol B, corchori fatty acid F, tianshic acid, mixture of dihydrovomifoliol-*O*-β-D-glucopyranoside and icariside B5, *p*-coumaric acid, tricin, 7,3′,5′-tri-*O*-methyltricetin 5-*O*-β-D-glucopyranoside, tricin-5-*O*-β-D-glucopyranoside, and 1-*O*-palmitoyl-3-*O*-(6-sulfo-α-D-quinovopyranosyl)-glycerol.	Govindan et al. 2018
Merostachys pluriflora	Leaves and culms	HPLC-DAD with authentic standards	Caffeic acid, ferulic acid, and vitexin.	Gagliano et al. 2018
Merostachys riedeliana	Leaves, culms and rhizomes	LC-DAD/ESI/MS/MS and GC-MS	Lactonic dimer of *p*-hydroxybenzoic acid, luteolin *C*-hexoside *O*-deoxyhexoside, luteolin *C*-pentoside *C*-hexoside, apigenin *O*-pentoside *C*-hexoside (C-8), apigenin *C*-hexoside *O*-pentoside, apigenin *C*-pentoside *C*-hexoside (C-6), luteolin 6-*C*-glucoside, apigenin 6-*C*-glucoside, 3,4-methylenedioxi mandelicacid, tricetintrimethyl ether 7-*O*-hexoside, benzoic acid, benzeneacetic acid, salicylic acid, *p*-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, vanillic acid, *p*-coumaric acid, protocatechuic acid, syringic acid, gallic acid, *m*-coumaric acid, vanillylmandelic acid, 4-methylmandelic acid, 3,4-methylenedioxymandelic acid, and *trans*-ferulic acid.	Jose et al. 2016.
Nastus elongatus	Shoots	Colorimetric methods	It was found variation in cyanogenic potential (HCNp).	Ballhorn et al. 2016
Oxytenanthera abyssinica	Leaves	Detection methods	Steroid glycoside, alkaloids, saponins, tannins, cardiac glycosides, flavonoids, phlobatanins, anthroquinone, and terpenes.	Bartholomew et al. 2013
Oxytenanthera abyssinica	Leaves	Detection methods	Polyphenols, flavonoids, saponins, general glycosides, coumarins, and cyanogenic glycosides.	Coffie et al. 2014

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Phyllostachys aurea	Leaves	GC-MS/FID	In leaf wax were identified fatty acids, primary alcohols, alkyl esters, aldehydes, alkanes, tocopherols, triterpenols, triterpenyl palmitates, and primary amides.	Racovita and Jetter 2016
Phyllostachys aureosulcata	Leaves	HPLC-UV	Scopoletin.	Wang et al. 2013
Phyllostachys aureosulcata. (=*Phyllostachys spectabilis*)	Leaves	HPLC-UV	Scopoletin.	Wang et al. 2013
Phyllostachys bambusoides	Leaves	HPLC	Isoorientin, orientin, and isovitexin.	Kumar et al. 2014
Phyllostachys bambusoides	Culms	LC-MS and NMR	Tachioside.	Li et al. 2008
Phyllostachys bambusoides	Leaves	HPLC-DAD-ESI-MS/MS	*p*-coumaric acid.	Zhao et al. 2017
Phyllostachys edulis	Leaves	HPLC-DAD	Isoorientin, vitexin, and isovitexin.	Xie et al. 2013
Phyllostachys edulis	Leaves	specified in Kweon et al. 2001	3-*O*-Caffeoyl-1-metylquinic acid.	Kweon et al. 2007
Phyllostachys edulis	Leaves	LC-MS/MS	Isoorientin.	Wedler et al. 2014
Phyllostachys edulis	Leaves	HPLC-UV and HPLC-MS	Orientin, isoorientin, vitexin, and isovitexin.	Yang et al. 2014
Phyllostachys edulis (=*Phyllostachys pubescens*)	Leaves, culms, rhizomes and roots	LC-MS-IT-TOF	Di-*C*,*C*-hexosyl apigenin, tricin derivative, *O*-hexosyl-*O*-deoxyhexosyl tricin, *O*-hexosyl tricin, 6-C-glucosyl apigenin (isovitexin), Di-*C*,*C*-hexosyl apigenin, *O*-hexosyl-deoxyhexosyl tricin, Di-*C*-glycosyl apigenin, chlorogenic acid, 8-*C*-glucosyl luteolin (orientin), 6-*C*-glucosyl luteolin (isoorientin), 8-*C*-glucosyl apigenin (vitexin), and *p*-courmaric acid.	Tanaka et al. 2014
Phyllostachys edulis (=*Phyllostachys pubescens*)	Shoots	LC-MS and NMR	Stigmasterol, dihydrobrassicasterol, and *β*-sitosterol.	Tanaka et al. 2013
Phyllostachys edulis (=*Phyllostachys pubescens*)	Leaves	HPLC	Isoorientin, orientin, vitexin, and isovitexin.	Sun et al. 2017
Phyllostachys edulis (=*Phyllostachys pubescens*)	Shoots	HPLC-UV	Protocatechuic, *p*-hydroxybenzoic, caffeic, chlorogenic, syringic, *p*-coumaric, and ferulic acids; catechin.	Park and Jhon 2010
Phyllostachys edulis (=*Phyllostachys pubescens*)	Leaves	not specified	Friedelin, isoorientin, *β*-sitosterol, tricin, and *p*-coumaric acid.	Choi et al. 2013
Phyllostachys edulis (=*Phyllostachys pubescens*)	Leaves	HPLC and HPLC-MS	Neochlorogenic, cryptochlorogenic, and chlorogenic acids; isoorientin, orientin, vitexin, isovitexin.	Zhu et al. 2018

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Phyllostachys glauca	Leaves	HPLC-DAD and LC-MS	Isoorientin, orientin, vitexin, isovitexin, luteolin-6-*C*-arabinoside, luteolin, and tricin.	Guo et al. 2013
Phyllostachys heterocycla	Culms	HPLC, HR-ESI-MS and NMR	Propiophenone 4'-*O*-primeveroside, 5-hydroxymethyl-2-furfural, 4-hydroxybenzoic acid, *trans-p*-coumaric acid, *trans*-ferulic acid, *N*,*N* -diferuloylputrescine, 4'-hydroxypropiophenone, *β*-arbutin, tachioside, isotachioside, 3,4'-dihydroxypropiophenone 3-*O*-glucoside, koaburaside, and (+)-lyoniresinol 9'-*O*-glucoside.	Yoshimura et al. 2017
Phyllostachys heterocycla	Shoots	HPLC, FT-IR and NMR	Polysaccharides were mainly composed of galactose, arabinose, xylose, and galacturonic acid.	Liu et al. 2018
Phyllostachys heterocycla cv. Pubescens	Leaves	GC-MS	Benzaldehyde, hexanoic acid; (Z)-5-octen-1-ol, benzyl alcohol, benzeneacetal dehyde, decalactone, citronellal, undecanal, phenylethyl alcohol, 4-ethyl-benzaldehyde, rose oxide, methyl salicylate, safranal, widdrol, farnesol, indole, cinerolone, *p*-acetylanisole, damascenone, vanillin, α-funebrene, α-ionone, cumaldehyde, caryophyllene oxide, isoeugenol, trans-geranylacetone, β-ionone, neocurdione, eugenol methyl ether, neoclovene, cedrol, geranyl isovalerate, octadecanal, trans-3-hexen-1-ol; 2,4-dimethyl-3-hexanol, 2,5-dimethyl-benzaldehyde, azulene, α-ionone, cashmeran, and dihydroactinidiolide.	Ming et al. 2015
Phyllostachys heterocycla cv. Pubescens	Leaves	not specified	Luteolin-6-*C*-neohesperidoside.	Duan et al. 2017

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Phyllostachys heterocycla cv. Pubescens	Leaves	GC-MS	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one, 6,10-dimethyl-5,9-undecadien-2-one, 1,1a,5,6,7,8-hexahydro-4a,8,8-trimethylcyclopropa[d]naphthalen-2(4aH)-one, 6,10,14-trimethyl-2-pentadecanone, 6,10,14-trimethyl-5,9,13-pentadecatrien-2-one, 6-heptyltetrahydro-2H-pyran-2-one, cedrol; β-eudesmol, α-eudesmol, 2-ethyl-1-dodecyl alcohol, 1-octadecanol, octanoic acid, *n*-nonylic acid, myristic acid, pentadecanoic acid, heptadecanoic acid, oleic acid, hexadecanoic acid, eicosane, 10-methyl-eicosane, tricosane, hexacosane, heptacosane, octacosane, triacontane, pentatriacontane, hexatriacontane, *n*-nonyl aldehyde, terpinyl acetate, 4-hydroxy-2-methyl acetophenone, 4,5,7,7a-tetrahydro-4,4,7a-trimethyl-2(6H)-benzofuranone, 1-octadecyne, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2,4–pentadienyl)-naphthalene, 9-eicosyne, 9-octadecenal, and 1-docosene.	Tao et al. 2017
Phyllostachys humilis	Leaves	HPLC-DAD; LC-MS/MS	Isoorientin, farobin A, *C*-pentosyl-luteolin, cassiaoccidentalin B, *O*,*C*-dideoxyhexosyl-luteolin, *O*-deoxyhexosyl-*C*-deoxyhexosylluteolin, tricin-5-*O*-glucoside, *O*-hexosyl-tricin, tricin, 5-feruloylquinic acid, sinapic acid derivative, caffeic acid, and sinapoyl hexose.	Van Hoyweghen et al. 2012
Phyllostachys nidularia	Leaves	HPLC-UV	Scopoletin.	Wang et al. 2013
Phyllostachys nigra	Leaves	Colorimetric methods, HPLC, NMR and LCQ-FLEET-HESI	*trans*-coniferyl alcohol, *p*-coumaric acid, *n*-feruloyl serotonin, caffeic acid ethyl ether, tricin, coumaryl alcohol, coumaric acid ethyl ether, and ferulic acid ethyl ether.	Shang et al. 2014
Phyllostachys nigra	Culms	HPLC and NMR	Fructose, glucose, sucrose, raffinose, stachyose, and maltotetraose.	Shin et al. 2016
Phyllostachys nigra	Leaves	HPLC-DAD and LC-MS-Q-TOF	Chlorogenic acid, isoorientin, orientin, isovitexin, salicylic acid, luteolin, tricin, and oxo-dihydroxy-octadecenoic acid.	Van Hoyweghen et al. 2014
Phyllostachys nigra	Leaves	HPLC-DAD; LC-MS/MS	*C*-hexosyl-*C*-pentosyl-apigenin, isoorientin, orientin, *O*-hexosyl-tricin, dihydroxybenzoic acid hexose, 5-feruloylquinic acid, coumaric acid conjugate, sinapoyl hexose, and sinapic acid derivative.	Van Hoyweghen et al. 2012
Phyllostachys nigra	Leaves	HPLC-UV	Scopoletin, umbelliferone, and coumarin.	Wang et al. 2013

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Phyllostachys nigra	Shoots	HPLC-UV	Protocatechuic, *p*-hydroxybenzoic, caffeic, chlorogenic, syringic, *p*-coumaric, and ferulic acids; catechin.	Park and Jhon 2010
Phyllostachys nigra	Leaves	HPLC-DAD-ESI/MS and NMR	Isoorientin, orientin, vitexin, luteolin 6-*C*-(6"-*O*-*trans*-caffeoylglucoside), vittariflavone, and tricin.	Kim et al. 2009
Phyllostachys nigra	Leaves	LC-MS and NMR	Isoorientin, orientin, vitexin, *cis*-coumaric acid, *p*-coumaric acid, luteolin 6-*C*-(6"-O-*trans*-caffeoylglucoside), vittariflavone, and tricin.	Jung et al. 2007
Phyllostachys nigra var. *henonis*	Leaves	HPLC-UV, IR, MS and NMR	Orientin, isoorientin, vitexin, and isovitexin.	Zhang et al. 2008
Phyllostachys nigra var. *henonis*	Leaves	UV, IR, MS and NMR	Tricin.	Jiao et al. 2007 (b)
Phyllostachys nigra var. *henonis*	Culms	EIMS	Friedelin.	Jiao et al. 2007 (a)
Phyllostachys nigra var. *henonis*	Leaves	HPLTC with authentic standards	Isoorientin, isovitexin, orientin, and vitexin.	Wang et al. 2010
Phyllostachys nigra var. *henonis*	Leaves and culms	HPLC-UV	Chlorogenic, caffeic, ferulic, and *p*-coumaric acids; orientin, isoorientin, vitexin, and isovitexin.	Gong et al. 2015
Phyllostachys nigra var. *henonis*	Culms	Colorimetric methods, HPLC and LC–Q–TOF-MS	Chlorogenic, caffeic, *p*-coumaric, and ferulic acids; luteolin, rutin, and catechin.	Choi et al. 2018
Phyllostachys propinqua	Leaves	HPLC-UV	Skimin, scopoletin, umbelliferone, and coumarin.	Wang et al. 2013
Phyllostachys sulphurea	Leaves	HPLC-UV	Scopoletin.	Wang et al. 2013
Pleioblastus argenteostriatus (= Sasa argenteostriata)	Leaves	HPLC-UV	Skimin, scopoletin, and umbelliferone.	Wang et al. 2013
Pleioblastus argenteostriatus (= *Sasa argenteostriata*)	Leaves	HPLC	Orientin, isoorientin, vitexin, isovitexin, *p*-coumaric, chlorogenic, caffeic, and ferulic acids.	Ni et al. 2012
Pleioblastus fortunei	Leaves	HPLC-UV	Scopoletin and umbelliferone.	Wang et al. 2013

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Pleioblastus fortunei (= Pleioblastus variegatus)	Leaves	HPLC-DAD and LC-MS/MS	*C*-hexosyl-*C*-pentosyl-apigenin, Di-*C*,*C*-pentosyl-apigenin, Iiovitexin, Di-*C*,*C*-pentosyl-apigenin, *O*-deoxyhexosyl-*C*-pentosyl-apigenin, isorientin, tricin, and sinapoyl hexose.	Van Hoyweghen et al. 2012
Pleioblastus kongosanensis f. *aureostriatus*	Leaves	HPLC-UV and Colorimetric methods	Chlorogenic, caffeic, *p*-coumaric, and ferulic acids; isoorientin, orientin, vitexin, and isovitexin.	Ni et al. 2013 (b)
Pseudosasa japonica	Leaves	HPLC-UV	Scopoletin and pimpinellin.	Wang et al. 2013
Pseudosasa japonica	Leaves	HPLC-DAD and LC-MS/MS	*C*-hexosyl-*C*-pentosyl-apigenin, *O*,*C*-dipentosyl-apigenin, Di-*O*,*C*-pentosyl-apigenin, Di-*C*,*C*-pentosyl luteolin, *O*-pentosyl-*C*-pentosyl luteolin, tricin-5-*O*-glucoside, *O*-hexosyl-*O*-deoxyhexosyl-tricin, *O*-hexosyl-tricin, tricin, sinapoyl hexose, and sinapic acid derivative.	Van Hoyweghen et al. 2012
Sasa borealis	Leaves	UV, IR, FAB-MS and NMR	Tricin 7-*O*-β-D-glucopyranoside, isoorientin, apigenin 6-*C*-β-D-xylopyranosil-8-*C*-β-D-glucopyranoside, and isoorientin 2-*O*-α-L-rhamnoside.	Park et al. 2007
Sasa kurilensis (= *Sasa coreana*)	Leaves	HPLC-DAD	Caffeic, ferulic, and *p*-coumaric acids; isoorientin, orientin, vitexin, isovitexin, hesperidin, naringin, and luteolin.	Yang et al. 2017
Sasa kurilensis var. *gigantea*	Leaves	HPLC-UV, MS and NMR	Kurilensin A and B; tricin-4'-*O*-β-D-glucopyranoside, and tricin-5-*O*-β-D-glucopyranoside.	Hasegawa et al. 2008
Sasa palmata	Leaves	GC-MS	DL-alanine, gluconic acid, phosphoric acid, ß-siosterol, β-amyrene, (6) α-amyrin acetate, and friedelin.	Zulkafli et al. 2014
Sasa quelpaertensis	Leaves and culms	HPLC-UV/Vis, FAB-MS and NMR	*trans*-*p*-coumaric acid.	An et al. 2008
Sasa quelpaertensis	Leaves	UV-Vis, IR and NMR	3-*O*-*p*-coumaroyl-1-(4-hydroxy- 3,5-dimethoxyphenyl)-1-propanone, 3-*O*-*p*-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-*O*-β-gulco- pyranosylpropanol, *N*-*p*-coumaroylserotonin, *N*-feruloylserotonin, and *p*-coumaric acid.	Sultana and Lee 2009
Sasa quelpaertensis	Leaves	HRFAB-MS, IR and NMR	*(E)*-3-hexenyl-β-glucopyranoside, 4-hydroxybenzoic acid, 3-hydroxy-1-(4-hydroxy 3,5-dimethoxyphenyl)-1-propanone; saikochromone A, tricin, tricin-7-*O*-glycoside, tricitin-3', 4', 5'-tri-*O*-methyl-7-*O*-β-glucopyranoside, isoorientin, daucosterol, and lutein.	Sultana and Lee 2010

Accepted name (= Synomyn used)	Part of plant	Analysis techniques	Identified and/or detected compounds	Reference
Sasa quelpaertensis	Leaves	HPLC	*p*-hydroxybenzoic, chlorogenic, *p*-coumaric, ferulic, sinapinic and cinnamic acids; rutin, taxifolin, myricetin, naringenin, rhamnetin, nobiletin, and tangeretin.	Herath et al. 2018 (b)
Sasa senanensis	Leaves	UV, HR-MS and NMR	Luteolin 6-*C*-β-D-glucoside, luteolin 7-*O*-β-D-glucoside, luteolin 6-*C*-α-L-arabinoside, and tricin.	Matsuta et al. 2011
Sasa veitchii	Leaves	HPLC-DAD, LC-MS-Q-TOF	Caffeoyl hexose, quinic acid, dihydrobenzoic acid, tricin, oxylipin, oxo-dihydroxy-octadecenoic acid, and trihydroxy-octadecenoic acid.	Van Hoyweghen et al. 2014
Sasa veitchii	Leaves	not specified	Arabinose, mannose, and galactose.	Sato et al. 2015
Sasa veitchii	Leaves	HPLC-DAD; LC-MS/MS	*C*-hexosyl-apigenin, Di-*O*,*C*-hexosyl-apigenin, Di-*O*,C-pentosyl-apigenin, *C*-hexosyl-*O*-pentosyl-luteolin, *O*-hexosyl-*C*-hexosyl luteolin, isorientin, Di-*C*,*C*-pentosyl luteolin, *O*-hexosyl-*C*-pentosyl-luteolin, tricin-5-*O*-glucoside, *O*-hexosyl-*O*-deoxyhexosyl-tricin, *O*-hexosyl-tricin, tricin, dihydroxybenzoic acid hexose, coumaroyl-quinic acid, coumaroyl hexose, and sinapoyl hexose.	Van Hoyweghen et al. 2012
Sasa veitchii *(= Sasa albo-marginata)*	Leaves	IR and NMR	*trans*-*p*-coumaric acid, vanilin, *p*-hydroxybenzaldehyde; 3-hydroxypyridine, and tricin.	Sakai et al. 2008
Sasa veitchii *(= Sasa albo-marginata)*	Leaves	HPLC	Tricin.	Akuzawa et al. 2011
Schizostachyum lumampao	Leaves	UV-Vis and Detection methods	Saponins, terpenoids, phenolics, tannins, and phytosterols.	Tongco et al. 2014
Shibataea chinensis	Leaves	Colorimetric methods	Chlorogenic, caffeic, ferulic, and *p*-coumaric acids; isoorientin, orientin, vitexin, and isovitexin.	Ni et al. 2013 (b)
Yushania brevipaniculata (= *Yushania chungii*)	Culms	HPLC-DAD, HPLC-ESI-MS, HPLC-MS	Methylchlorogenic acid, *p*-OH-cinnamic acid, vicenin, luteolin derivative, isoorientin rhamnoside, and apigenin derivative.	Keski-Saari et al. 2008

4. Biological potential of bamboos

The antioxidant capacity of bamboo extracts was the most-tested biological activity, representing 58% of the total papers found (Figure 8; Table 3). Bamboos have a greater abundance of phenolic compounds, being justified the high number of studies testing the the antioxidant potential of these species.

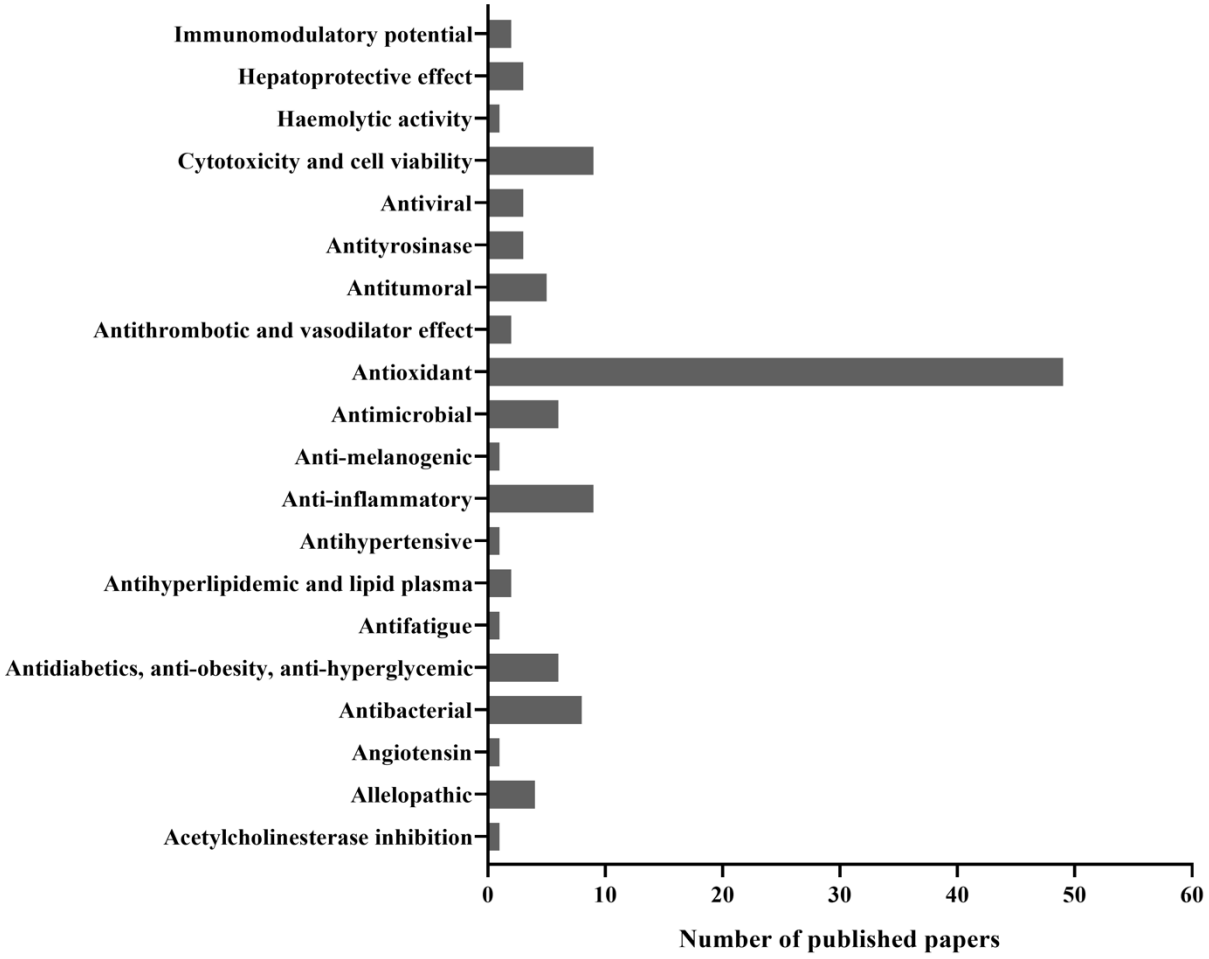


Figure 8 – Number of studies from 2007-2018 according to the biological activity tested.

Flavonoids were the most reported phenolic class in bamboo especies, and their structural chemistry is ideal for free radical-scavenging activities. Wang et al. (2018) concluded that a C2=C3 double bond, a 4-carbonyl group, and a hydroxylation patterns especially on C3 and a catechol moiety on B-ring are the major structural features conferring high biological activities.

Phyllostachys nigra has been the most tested species regarding its biological activities. Its leaf extract is a potent inhibitor of NF-kB-induced gene expression, showing *in vitro* anti-inflammatory activity (Van Hoyweghen et al. 2014). In a clinical study, it was observed that women that consumed shoots of this species had a significant decrease in serum total cholesterol,

low-density lipoprotein cholesterol (HDL), and the atherogenic index compared with the dietary fiber-free diet (Park and Jhon 2009). This result suggests an anti-cholesterol potential of this species.

A large number of studies using *Phyllostachys* might be attributed to the fact that in China, a product known as AOB (antioxidant bamboo extract) is made from a mixture of leaves from different Asian bamboo species, including *P. nigra*. This product was approved by the Chinese Food Additive Standardization Committee as a novel natural antioxidant and it is used as an additive in different food products (Zhang et al. 2005).

DPPH was the most popular antioxidant method used (Figure 9; Table 3). This method is widespread as antioxidant screening because it is technically a simple and fast assay, needing only a spectrophotometer as instrument for its analysis (Karadag et al. 2009).

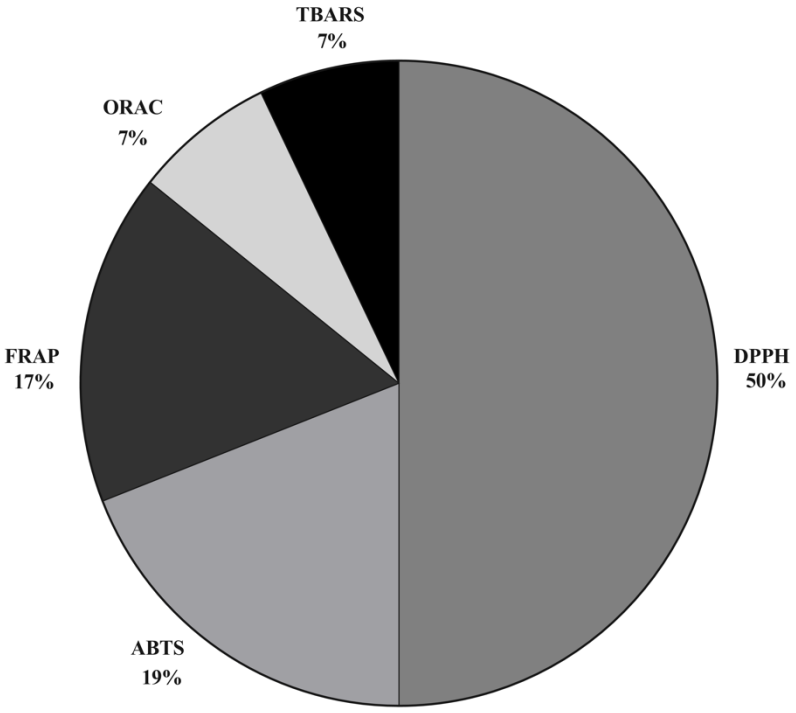


Figure 9 – Number of studies from 2007-2018 according to the antioxidant method used.

Bamboo species also showed *in vivo* biological potential (Table 3). In a comparative *in vivo* study conducted in Wistar albino rats, *Bambusa arundinacea* (Retz.) Willd. aqueous leaf extract showed higher antithrombotic activity compared to phytomenadione (Vitamin K) (Abirame et al. 2018).

Administration of *Bambusa balcooa* Roxb. aqueous extract in alloxan-induced diabetic rats showed a significant reduction in fasting blood glucose and in glycated hemoglobin, while

plasma insulin level was elevated when compared with the diabetic control group (Goyal et al. 2017).

In groups of rats treated with hydroethanolic leaf extract of *Dendrocalamopsis oldhami* (Munro) Keng F., a significant decrease in superoxide dismutase and glutathione peroxidase activities in liver and kidneys was observed, and a significant decrease in catalase activity in the kidneys. Also, a significant decrease was observed in the quantities of thiobarbituric acid in the liver and kidneys of the treated groups compared with the control group (Lv et al. 2012).

The leaf ethyl acetate fraction of *Phyllostachys bambusoides* Siebold & Zucc was orally administrated in mice and it was observed that this extract acts as an effective immunostimulator eliciting for both Th1 and Th2 immune responses, suggesting an *in vivo* immunomodulatory potential of this species (Kumar et al. 2014).

Although it can be found several papers reporting these main biological activities, different extracts were used, as ethanol, hydromethanol, ethyl acetate, and hexane extracts, but few papers report isolated compounds and their dose-response, bioavailability or toxicity.

Table 3 –Bamboo biological activities reported from 2007 to 2018.

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Arundinaria gigantea	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Aulonemia aristulata	Leaves	Allelopathic	Allelopathic influence on the germination of seeds of lettuce and *Sesbania virgate.*	Grombone-Guaratini et al. 2009
Bambusa arundinaceae	Leaves	Antimicrobial and Haemolytic activity	Disc diffusion and Resazurin microtitre-plate for antimicrobial assay; heamolytic activity against human blood erythrocytes.	Zubair et al. 2013
Bambusa arundinaceae	Shoots	Total phenolic and flavonoid content; antioxidant and anti-inflammatory	Folin-Ciocalteu, aluminium chloride, sodium phosphate-ammonium molybdate, DPPH, alkaline DMSO, hydrogen peroxide scavenging, and lipid peroxidation assays; protein denaturation and HRBC membrane stabilization methods were used to assess *in vitro* anti-inflammatory activity.	Vanitha et al. 2016
Bambusa arundinaceae	Leaves	Antithrombotic activity	Comparative *in vivo* study.	Abirame et al. 2018
Bambusa balcooa	Leaves	Total phenol and flavonoid content; antioxidant and anti-hyperglycemic activity	DPPH, FRAP, scavenging of hydrogen peroxide, and TBARS; Swiss albino mice were used to assess acute toxicity test; Alloxan-induced Wistar albino rat model was used to evaluate *in vivo* anti-hyperglycemic activity; Blood glucose and biochemical parameters (Glutathione peroxidase, superoxide dismutase, and malondialdehyde) were performed to evaluate *in vivo* anti-hyperglycemic activity.	Goyal et al. 2017
Bambusa balcooa	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Bambusa bambos	Leaves	Estrogenic effects	Growth of MCF-7 cells *in vitro*.	Sriraman et al. 2015
Bambusa bambos	Seeds	Effect on metabolic symptoms of experimentally induced polycystic ovarian disease	The female Wistar rat model with Letrozole-induced polycystic ovarian disease was used to evaluate the effects of bamboo seed oil on metabolic symptoms of polycystic ovarian disease; Biochemical parameters (glucose, total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides levels) and the reproductive system of female rats were assessed.	Soumya et al. 2016
Bambusa bambos (= *Bambusa bambose*)	Leaves	Antibacterial	Agar well diffusion method on bacterial strains like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp. *Citrobacter* spp., *Acinetobacter* spp. *Streptococcus* spp., *Enterobacter* spp., and *Proteus mirabilis*.	Wasnik and Tumane 2014

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Bambusa emeiensis (= Neosinocalamus affinis)	Leaves	Antioxidant	DPPH, ABTS, superoxide anion, and NO radicals.	Luo et al. 2015
Bambusa emeiensis (= Sinocalamus affinis)	Shoots	Total flavonoid	Not specified.	Hu et al. 2018
Bambusa nutans	Leaves	Antioxidant	Folin-Ciocalteu and DPPH.	Tripathi et al. 2015
Bambusa nutans	Leaves	Total phenol and flavonoid content, antioxidant and alpha-glucosidase inhibitory activity	Folin-Ciocalteu, Aluminum chloride, DPPH, reducing power, hydroxyl radical scavenging, metal chelating assay, and Alpha-Glucosidase inhibition assay.	Pande et al. 2018
Bambusa polymorpha	Shoots	Total phenol and ascorbic acid content; oxidative stability and microbiological evaluation	Folin-Ciocalteau method was used to determine total phenolic contents; oxalic acid, metaphosphoric, H_2SO_4, and ammonium molybdato were used to determine ascorbic acid content; the TBARS (thiobarbituric acid reacting substances) number was used to evaluate oxidative stability of products; ready-made media were used for couting of different microorganisms.	Thomas et al. 2016
Bambusa rutila	Leaves	Antioxidant	Superoxide radical system assay and hydroxyl radical scavenging assay.	Gao et al. 2012
Bambusa textilis	Leaves	Antioxidant, anti-obesity activity, total phenol and flavonoid content	DPPH, FRAP, and *β*-carotene bleaching assays were used to determine *in vitro* antioxidant effect; High-fat diet male Sprague-Dawley rat model was used to determine *in vivo* antioxidant and anti-obesity activities; Activity of superoxide dismutase, glutathione peroxidase, and inhibition of lipid peroxidation in the serum and livers of rats were performed to evaluate *in vivo* antioxidant activity; Total cholesterol, triacylglycerol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol levels in the serum and body and liver weights were analysed to evaluate *in vivo* anti-obesity activity; Folin-Ciocalteau and aluminium nitrate-sodium nitrite was used to determine total phenolic and flavonoid contents.	Liu et al. 2016
Bambusa tulda	Leaves	Total phenol and flavonoid content and antioxidant	Folin-Ciocalteau, aluminum chloride, DPPH, reducing power, hydroxyl radical scavenging, and metal chelating assays.	Pande et al. 2017

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Bambusa tulda	Leaves	Osteogenic differentiation and mineralization of human mesenchymal stem cells.	Alkaline phosphatase activity assays and Alizarin red S staining were performed to evaluate the osteogenic differentiation of stem cells.	Lee et al. 2018
Bambusa tuldoides	Culms	Antioxidant	DPPH and FRAP.	Jia Sun et al. 2013 (a)
Bambusa vulgaris	Leaves	Antimicrobial	Agar diffusion method using *Aspergillus niger*, *Verticillium alboatrum*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae.*	Owolabi and Lajide 2015
Bambusa vulgaris	Leaves	Antidiabetic	Blood glucose levels in Streptozotocin induced diabetic rats.	Senthilkumar et al. 2011
Bambusa vulgaris	Leaves	Antioxidant	Folin-Ciocalteu and DPPH.	Tripathi et al. 2015
Bambusa vulgaris var. *vittata*	Leaves	Total phenol and flavonoid content and antioxidant	Folin Ciocalteu, aluminium chloride; DPPH; FRAP; hydrogen peroxide scavenging.	Goyal et al. 2013
Chimonobambusa quadrangularis	Shoots	Antioxidant	DPPH, ABTS, hydroxyl radical scavenging, and metal chelating activity.	Chen et al. 2018
Chimonobambusa quadrangularis	Culms	Antioxidant	DPPH, ORAC, ABTS, hydroxyl radical scavenging, reducing power, and iron chelating assays.	Zhang et al. 2018
Dendrocalamopsis oldhami	Leaves	Antioxidant	*In vivo*: Measuring superoxide dismutase, catalase, glutathione peroxidase, and the values of thiobarbituric acid reactive substances activities. *In vitro*: FRAP and DPPH.	Zhao-Lin et al. 2012
Dendrocalamus hamiltonii	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Dendrocalamus hamiltonii	Shoots	Regulating insulin sensitivity in mice	Mice were fed a low-fat diet or a high-fat diet with 10% fiber as cellulose, or bamboo shoot fibers, *D. hamiltonii* fibers for 13 weeks. Biochemical analysis: Glucose and insulin tolerance tests, Insulin challenge and western blotting.	Li et al. 2018
Dendrocalamus latiflorus	Shoots	Regulating insulin sensitivity in mice	Mice were fed a low-fat diet or a high-fat diet with 10% fiber as cellulose, or bamboo shoot fibers, *D. hamiltonii* fibers for 13 weeks. Biochemical analysis: Glucose and insulin tolerance tests, Insulin challenge and western blotting.	Li et al. 2018

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Dendrocalamus strictus	Leaves	Total phenol and flavonoid content; and antioxidant	Folin Ciocalteu, aluminium chloride; DPPH, FRAP, and hydrogen peroxide.	Goyal et al. 2011
Dendrocalamus asper	Shoots	Response was tested on Kv1.4 potassium channel	The response was tested on Kv1.4 potassium channel which was injected into viable oocytes that was extracted from *Xenopus laevis*. The current was detected by the two-microelectrode voltage clamp, holding potential starting from −80 mV with 20 mV step- up until +80 mV. Readings of treatments with 0.1% DMSO, 4-hba concentrations and K channel blockers were taken at +60 mV.	Jingli et al. 2018
Dinochloa scandens	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Fargesia robusta	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Fargesia robusta	Leaves	Antioxidant	ABTS (TEAC) and ORAC.	Van Hoyweghen et al. 2010
Fargesia rufa	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Gigantochloa levis	Leaves	Total phenolic content and antioxidant	Folin-Ciocalteu and DPPH.	Tongco et al. 2016
Gigantochloa ligulata	Leaves	Total phenol, antioxidant and antityrosinase	Folin-Ciocalteu, Ferric thyocianate (FTC), Xanthin/Xanthine oxidase superoxide scavenging activity (X/XOD), DPPH, and Tyrosinase inhibitory.	Ilham et al. 2008
Gigantochloa scortechinii	Leaves	Total phenol, antioxidant and antityrosinase	Folin-Ciocalteu, Ferric thyocianate (FTC), Xanthin/Xanthine oxidase superoxide scavenging activity (X/XOD), DPPH, Tyrosinase inhibitory.	Ilham et al. 2008
Guadua amplexifolia	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Guadua angustifolia	Culms	Total phenol and flavonoid content; and antioxidant	Folin-Ciocalteu, Aluminiun chloride, DPPH, and ABTS.	Mosquera et al. 2015
Guadua angustifolia	Leaves	Total phenol content and antioxidant	Folin-Ciocalteu and DPPH.	Álvarez et al. 2015
Guadua angustifolia	Leaves	Antioxidant	DPPH.	Valencia et al. 2011

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Indocalamus latifolius	Leaves	Antibacterial	Filter agar-disk diffusion method against four bacterial strains: *Staphylococcus aureus*; *Escherichia coli*; *Bacillus thuringiensis*; *Pseudomonas solanacearum*.	Jia Sun et al. 2015 (b)
Indocalamus latifolius	Leaves	Antioxidant	DPPH and FRAP.	Ni et al. 2013 (a)
Melocanna bacífera	Leaves	Phyto-prophylactic properties against low pH stress and saprolegniasis in *Labeo rohita* (rohu).	Cumulative mortality rates of Labeo rohita (rohu) fingerlings, infected with zoopores of Saprolegnia parasitica, fed with supplemented diet with Bamboo Leaf Alcoholic (BLAL) extract and with diet deprived of BLAL.	Khan et al. 2018
Melocanna bacífera	Fruits	Total phenol content	Folin-Ciocalteau.	Govindan et al. 2016
Melocanna bacífera	Fruits and leaves	Cytotoxicity and acetylcholinesterase inhibition assay	Cytotoxicity against three cancer cell lines (SIHA, MCF 7 and C6) and the non-cancerous cell line (MCF 10A) by MTT assay; and acetylcholinesterase (AChE) inhibition assay.	Govindan et al. 2018
Merostachys pluriflora	Leaves, culms and rhizomes	Allelopathic	Allelopathic influence on the germination of seeds of tomato and rice.	Faria and Grombone-Guaratini 2011
Merostachys pluriflora	Leaves and culms	Antioxidant	DPPH, FRAP, ABTS, ICR, and ORAC.	Gagliano et al. 2018
Merostachys riedeliana	Leaves, culms and rhizomes	Allelopathic	Inhibitory effects on seed germination and seedlings growth of seeds of *Solanum lycopersicum* L. (tomato), *Orysa sativa* L. cultivar BRS (rice), *Erythrina verna* Vell., and *Mimosa bimucronata* (DC.) Kuntze.	Jose et al. 2016
Merostachys skvortzovii	Leaves	Allelopathic	Allelopathic influence on the germination of seeds of *Mimosa scabrella* Benth	Sanquetta et al. 2013
Oxytenanthera abyssinica	Leaves	Antioxidant	DPPH, FRAP, TBARS.	Bartholomew et al. 2013
Phyllostachys aureosulcata f. *aureocaulis*	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys aureosulcata	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys aureosulcata f. *spectabilis*	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Phyllostachys bambusoides	Leaves	Immunomodulatory potential	Acute toxicity, Immunization schedule, Haemagglutination antibody (HA) titre, DTH reaction, Splenocyte proliferation assay, Macrophage function assay (NO production), *In vivo* carbon clearance and *Candida albicans* clearance, Determination of IFN-γ, IL-2 and IL-4 by the ELISA method, Lymphocyte phenotyping in spleen.	Kumar et al. 2014
Phyllostachys bambusoides	Culms	Lipid plasma levels; antioxidants and anti-inflammatory	Kits of colorimetric methods (Lipid assay); TEAC; TBARS; Hepatic protein carbonyl spectrophotometry assay; SOD, Catalase, GSH-px, GSH reductase; eletrophoretic mobility shift assay. Nuclear binding activity of NFkβ (anti-inflammatory).	Lee et al. 2008
Phyllostachys bambusoides	Whole bamboo	Protective effect against neuronal damage; anti-plasmin effects	NMDA induced cell death in cortical neurons with MTT; fibrin and fibrinogen degradations products (FDP).	Hong et al. 2010
Phyllostachys bambusoides	Culms	Antioxidant	DPPH and TEAC.	Li et al. 2008
Phyllostachys bambusoides	Leaves	Cell viability and inhibition of adipogenesis	MTT assay was used to determine cell viability; measurement of glycerol and triglyceride contents and expression levels of adipogenic transcription factors (PPARγ, C/EBPα, SREBP-1c) and enzymes (FAZ, ACC, p-ACC, and AMPK) in 3T3-L1 adipocytes were used to evaluate inhibition of adipogenesis.	Kwon et al. 2017
Phyllostachys bissetii	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys edulis	Leaves and culms	Protective effect of lipotoxicity	Prevention of lipoapoptosis, cytotoxicity induced by Palmitic acid in cells.	Panee et al. 2008
Phyllostachys edulis	Leaves and culms	Total phenol and flavonoid content, antitumoral, and antioxidants	Folin-Ciocalteu, Aluminum Chloride; *in vivo*: Total Glutathione-S-transferase (GST), Total UGT , Total Sulfotransferase (SULT), FRAP and ABTS activities; Antitumoral of breast cancer cells.	Lin et al. 2008
Phyllostachys edulis	Leaves	Photochemopreventive of UVA-mediated apoptosis (antitumoral)	Photochemopreventive of UVA-mediated apoptosis in immortalized HaCaT keratinocites.	Kweon et al. 2007

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Phyllostachys edulis	Leaves	Cell viability assay, anti-inflammatory, and cell migration assay	MTT, anti-inflammatory effects on tumor necrosis factor alpha-induced overproduction of interleukin 8, vascular endothelial growth factor, and interleukin 6 in immortalized human keratinocytes and wound-healing effects were evaluated in 3T3-swiss albino mouse fibroblasts.	Wedler et al. 2014
Phyllostachys edulis	Leaves	Starch digestion	Measurement and kinetics assay of α-amylase inhibitory activity; thermal stability; molecular modeling.	Yang et al. 2014
Phyllostachys edulis	Leaves	Antitumoral	The growth of human osteosarcoma cell lines 143B and MG-63 and lung fibroblast MRC-5 cells was determined by MTT assay. Apoptosis was demonstrated using TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay and flow cytometric analysis. Phosphorylation and protein levels were determined by immunoblotting.	Chou et al. 2014
Phyllostachys edulis	Culms	Anti-inflammatory	HIV-1 transgenic (TG) rat model was used to assess in vivo anti-inflammatory effect. Gene and protein expression of interleukin 1 beta (IL-1β), glial fibrillary acidic protein (GFAP), and ionized calcium-binding adapter molecule 1 (Iba1) and protein expression of p65 and c-Jun were performed to evaluate *in vivo* anti-inflammatory activity.	Pang and Panee 2016
Phyllostachys edulis (= Phyllostachys pubescens)	Leaves, culms, rhizomes and roots	Antioxidant, antibacterial, anti-allergy, melanin biosynthesis, cell viability, determination of melanin content, immunoglobulin E (IgE) production	ABTS, ORAC, and SOD; Broth dillution method against *S. aureus* Melanoma cells B16; and MTT	Tanaka et al. 2014
Phyllostachys edulis (= Phyllostachys pubescens)	Leaves and culms	Antibacterial	Broth dillution method against *Escherichia coli.*	Afrin et al. 2012
Phyllostachys edulis (= Phyllostachys pubescens)	Shoots	Antibacterial	Micro dillution method against *Staphylococcus aureus* and *Escherichia coli.*	Tanaka et al. 2013
Phyllostachys edulis (= Phyllostachys pubescens)	Leaves	Antioxidant	On-line DPPH and ABTS.	Sun et al. 2017

Accepted name (= Synomyn used)	**Part of plant**	**Analyses**	**Methodology**	**Reference**
Phyllostachys edulis (= Phyllostachys pubescens)	Shoots	Antioxidant; antimicrobial activity and angiotensin converting enzyme (ACE) inhibition activity	DPPH; paper disc method against *E. coli*, *Ent. faecium*, *Ent. faecalis*, S. *mutans* was used to antimicrobial; the angiotensin-converting enzyme inhibition activity was measured spectrophotometrically using Hip-His-Leu.	Park and Jhon 2010
Phyllostachys edulis (= Phyllostachys pubescens)	Shoots	Antibacterial	Broth dillution and microbroth dillution method against *Staphylococcus aureu*s.	Tanaka et al. 2011
Phyllostachys edulis (= Phyllostachys pubescens)	Whole bamboo	Protective effect against neuronal damage and anti-plasmin effects	NMDA induced cell death in cortical neurons with MTT; fibrin and fibrinogen degradations products (FDP).	Hong et al. 2010
Phyllostachys edulis (= Phyllostachys pubescens)	Leaves	Anti-inflammatory	Measurement of reactive oxygen species (ROS) Intracellular; Monocyte-endothelial cell adhesion assay; IL-6 ELISA; Immunoblotting Cells. Anti-inflammatory activity on tumor necrosis factor-alpha (TNF-α)-induced monocyte adhesion in human umbilical vein endothelial cells.	Choi et al. 2013
Phyllostachys edulis (= Phyllostachys pubescens)	Leaves	Antioxidant	DPPH	Zhu et al. 2018
Phyllostachys flexuosa	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys heterocycla	Culms	Antibacterial	Standard two-fold dilution method was used to assess antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.	Yoshimura et al. 2017
Phyllostachys heterocycla	Shoots	Hypoglycemic activity	The hypoglycemic activity of polysaccharides was evaluated by Caco-2 monolayer cells model *in vitro*.	Liu et al. 2018
Phyllostachys heterocycla cv. *pubescens*	Leaves	The effect of VOCs on environmental health	The effect of VOCs on environmental health was evaluated by analyzing the metabolic indices of the type 2 diabetic mouse model.	Ming et al. 2015

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Phyllostachys heterocycla cv. *pubescens*	Leaves	Antifatigue activity	Rat model undergoing the weight-loaded forced swimming test was used to evaluate antifadigue activity. Histological analysis of the liver and skeletal muscle, biochemical parameters of serum and tissues (blood urea nitrogen, lactate dehydrogenase, plasma lactic acid, liver glycogen, skeletal muscle glycogen, aspartate aminotransferase, alanine aminotransferase, reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase, glutathione peroxidase, TNF-α, IL-1β, IL-6, and IL-10 levels), semiquantitative RT-PCR analysis for Nrf2 and HO-1, and western blot analysis for Nrf2 and HO-1 were performed to evaluate antifadigue activity.	Duan et al. 2017
Phyllostachys heterocycla cv. *pubescens*	Leaves	Antimicrobial	Double-plate punching method was used to evaluate antimicrobial activity against *Bacillus subtilis*, *Flavobacterium*, *Escherichia coli*, *Pseudomonas fluorescens*, and yeast *Saccharomyces cerevisiae.*	Tao et al. 2017
Phyllostachys humilis	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Phyllostachys humilis	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys iridescens	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys mannii	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys nigra	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Phyllostachys nigra	Leaves	Anti-inflammatory	COX inhibitor screening assay.	Van Hoyweghen et al. 2014
Phyllostachys nigra	Leaves	Total phenol and flavonoid content and antioxidant	Folin-Ciocalteu, Alluminum Chloride, DPPH, HPLC-ABTS+.	Shang et al. 2014
Phyllostachys nigra	Leaves	Total phenol content; antioxidant; and digestive stability and bioaccessibility assessment	Folin-Ciocalteau; DPPH and ABTS; *in vitro* digestion system (using α-amylase, pancreatic lipase, pepsin, pancreatin, and bile extract) and HPLC were used to evaluate digestive stability and bioaccessibility of tricine and extract of the digestates of each digestion phase.	Shang et al. 2016

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Phyllostachys nigra	Culms	Cell viability assay; cell migration assay; ex vivo aorta ring sprouting assay; ICAM-1 promoter-reporter assay; NF-κB-dependent transcriptional activity; and cell adhesion assay	MOVAS-1 vascular smooth muscle cells and Cell Counting Kit-8^{TM} were used to evaluate cell viability; MOVAS-1 cells and platelet-derived growth factor composed of two B subunits (PDGF-BB) were used to assess wound healing cell migration; thoracic aortas from male Balb/c mice were used to detemine formation of aorta ring sprouting; MOVAS-1 cells, pRL-null plasmid encoding Renilla luciferase, TNF-α, and Dual-Glo Luciferase Assay System were used to assess TNF-α-induced ICAM-1 expression and NF-κB-dependent transcriptional activity; red fluorescent BCECF-labeled THP-1 monocytes, MOVAS-1 cells, and TNF-α were used to evaluate cell adhesion.	Shin et al. 2016
Phyllostachys nigra	Shoots	Antioxidant; antimicrobial activity and angiotensin converting enzyme (ACE) inhibition activity	DPPH; paper disc method against *E. coli*, *Ent. faecium*, *Ent. faecalis*, *S. mutanswas* used to antimicrobial; the angiotensin-converting enzyme inhibition activity was measured spectrophotometrically using Hip-His-Leu.	Park and Jhon 2010
Phyllostachys nigra	Leaves	Antioxidant	ABTS and DPPH.	Kim et al. 2009
Phyllostachys nigra	Shoots	Blood glucose, lipid profile and liver function	Clinical study of healthy woman 21 to 23 years old, by blood sampling analysis and fecal excretion.	Park and Jhon 2009
Phyllostachys nigra	Leaves	Antidiabetics complications (Cataract) and Antioxidant	Aldose Reductase (ARL2) Raty Lens; Advnace Glycation and Products (AGE); Photochemiluminescence.	Jung et al. 2007
Phyllostachys nigra	Whole bamboo	Protective effect against neuronal damage and anti-plasmin effects.	NMDA induced cell death in cortical neurons with MTT; fibrin and fibrinogen degradations products (FDP).	Hong et al. 2010
Phyllostachys nigra var. *henonis*	Leaves	Antitumoral	MTT; nitroblue tetrazolium and Flow citometry; antitumoral leukemia cells (HL-60).	Kim et al. 2007
Phyllostachys nigra var. *henonis*	Culms	Antihyperlipidemic, antihypertensive and vasodilator effect	Blood lipid assay; Blood pressure of Spontaneous hypertensive rats; tension measurements of rat's aorta vasoconstrited by phenylephrine.	Jiao et al. 2007 (a)

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Phyllostachys nigra var. *henonis*	Culms	Antimicrobial	Agar diffusion method against *S. aureus; B. subtilis; E. coli; A. niger; P. citrinum; S. cerevisiae*.	Zhang et al. 2010
Phyllostachys nigra var. *henonis*	Leaves	Total phenol and flavonoid content, and antioxidant.	Total flavonoids and total polyphenols were determined by a photocolorimetric method by comparison with authentic standards, DPPH, superoxide anion radical, hydroxyl radical, hydrogen peroxide radical.	Gong et al. 2015
Phyllostachys nigra var. *henonis*	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys nigra var. *henonis*	Culms	Anti-melanogenic and antioxidant	*In vitro* tyrosinase inhibition activity, *in vivo* cytotoxic, DPPH, ABTS, and hydroxyl radical scavenging.	Choi et al. 2018
Phyllostachys nigra var. *nigra*	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys parvifolia	Leaves	Protective effect against the ionizing radiation induced genetic damage; and total flavonoid content	*In vitro* cytokinesis blocked micronuclei assay, using human peripheral blood lymphocytes, was used to evaluate protective effect against the ionizing radiation induced genetic damage; aluminum chloride method was used to determine total flavonoid content.	Patel et al. 2016
Phyllostachys sulphurea var. sulphurea	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys violascens	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys viridiglaucescens	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Phyllostachys vivax f. *aureocaulis*	Shoots	Total phenol content and antioxidant	Folin-Ciocalteu and FRAP.	Neményi et al. 2015
Pleioblastus kongosanensis f. *aureostriatus*	Leaves	Total phenol, flavonoid and terpenoid content. ROS scavenging activity and DNA damage prevention ability.	Folin-Ciocalteu, aluminum nitrate–sodium nitrite, Vanillin–glacial acetic acid, ROS scavenging activities and inhibitory effects of PLE and fractions on DNA oxidative damage were assayed by chemiluminescence methods (CL).	Ni et al. 2013 (c)

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Pleioblastus kongosanensis f. *aureostriatus*	Leaves	Total phenol, flavonoid, and terpenoids content; and antioxidant	Folin–Ciocalteu, Aluminum nitrate-sodium nitrite, Vanillin-glacial acetic acid, DPPH and FRAP.	Ni et al. 2013 (b)
Pleioblastus fortunei *(= Pleioblastus variegatus)*	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012
Pseudosasa japonica	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al., 2012
Sasa albo-marginata	Leaves	Antiviral	Anti-human cytomegalovirus (HCMV) activity in the human embryonic fibroblast cell line MRC-5 by plaque assay and western blot.	Sakai et al. 2008
Sasa albo-marginata	Leaves	Antiviral	Anti-human cytomegalovirus (HCMV) activity in the human embryonic fibroblast cell line MRC-5 by plaque assay and western blot.	Akuzawa et al. 2011
Sasa argenteastriatus	Leaves	Total phenol, flavonoid and triterpenes content; and antioxidants	Folin-Ciocalteu, aluminum nitrate, vanillin-acetic acid solution, DPPH and FRAP	Ni et al. 2012
Sasa borealis	Leaves	Total phenol and flavonoid content, antioxidants and antimicrobial	Folin-Ciocalteu; aluminium chloride; DPPH, ABTS, FRAP, Ferrous ion chelating effect; antimicrobial micro-dilution test.	Oh et al. 2013
Sasa borealis		Anti hiperglicemia apoptosis in Human Umbilical Endothelial cells (HUVEC). Treatment of diabetes endothelial dysfunction	Measurement of ROS production by fluorescence; Flow citometry evaluation of early apoptosis; Immunocitochemistry and Measurement of Peroxynitrite anion by fluorescence.	Choi et al. 2008
Sasa borealis	Leaves	Antioxidant and cytoprotective	DPPH and t-BOOH-induced oxidative damage in HepG2 cells.	Park et al. 2007

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Sasa kurilensis (= Sasa coreana)	Leaves	Cell viability assay; anti-inflammatory, antiadipogenic activity; and functional macrophage migration assay	*In vitro* anti-inflammatory activity was evaluated through of nitrite production assay and RT-PCR analysis. *In vitro* antiadipogenic effect was assessed using Red Oil O assay. Anti-inflammatory and antiadipogenic activities were also evaluated by Immunoblot analysis. *In vitro* chemotaxis assay was used to evalute functional interaction between macrophages and adipocytes exposed to the extract. MTT assay was used to evaluate cell viability of RAW 264.7 and 3T3-L1 cells.	Yang et al. 2017
Sasa kurilensis var. *gigantea*	Leaves	Antioxidant	DPPH.	Hasegawa et al. 2008
Sasa palmata	Leaves, culms, rhizomes and roots	Antioxidant	DPPH.	Kurosumi et al. 2007
Sasa palmata	Leaves	Total phenol and antioxidant	Folin-Ciocalteu and DPPH.	Zulkafli et al. 2014
Sasa quelpaertensis	Leaves	Antiviral	Porcine reproductive and respiratory syndrome virus infection in cultured porcine alveolar macrophages.	Kang and Lee 2015
Sasa quelpaertensis	Leaves	Measurement of body weight, food intake, and organ weights; biochemical analysis; histological examination of liver; western blot analysis; RNA extraction and real-time PCR	All procedures were performed using commercial kits according to the manufacturer's instructions.	Kim et al. 2014
Sasa quelpaertensis	Leaves and culms	Cell viability assay; melanogenesis and tyrosinase expression	MTT; murine melanoma cells melanin production measured by spectrophotometer; melanoma cell lysates tyrosinase assay.	An et al. 2008
Sasa quelpaertensis	Leaves	Tyrosinase inhibition assay	Colorimetric.	Sultana and Lee 2009
Sasa quelpaertensis	Leaves	Antioxidant	DPPH.	Sultana and Lee 2010
Sasa quelpaertensis	Leaves	Anti-inflammatory	NO production; NF-kB activation in LPS-stimulated RAW 264.7 cells.	Hwang et al. 2007

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Sasa quelpaertensis	Leaves and culms	Total Phenol and flavonoid content; Antioxidant and anti-inflammatory	Folin-Ciocalteu; aluminium chloride; DPPH, ABTS; for the anti-inflammatory assay was used RAW 264.7 murine macrophage cell line.	Ko et al. 2018
Sasa quelpaertensis	Leaves	Hepatoprotective effect and antioxidant	HepG2 cells, human liver epithelial-like monolayer hepatoblastoma cells, were used for *in vitro* assays. MTT and colony formation assay to measure cytotoxicity and cytoprotective effect of SQE in ethanol exposed HepG2 cells. Production of intracellular ROS was evaluated using 2,7-dichloro-fluorescein diacetate (DCF-DA) assay. Griess assay. Western blot. Propidium iodide (PI) staining assay. Binge drinking model was used for *in vivo* evaluation of SQEE80's hepatoprotective effect. Tissue processing and hematoxylin-and-eosin (H&E) staining. Measurement of serum ethanol content. Lipid peroxidation assay in liver. Measurement of GSH level in liver. Immunohistochemical staining.	Herath et al. 2018 (a)
Sasa quelpaertensis	Leaves	Cytotoxicity and hepatoprotective effect	*In vitro* Cell viability measurement by MTT; Propidium iodide (PI) staining assay; In vivo experiment performed using binge alcohol consumption model; Histopathological analysis of liver; Measurement of blood alcohol content; Thiobarbituric acid reactive substance (TBARS) assay; Determination of hepatic glutathione (GSH); Immunohistochemical staining; Western blotting.	Herath et al. 2018 (b)
Sasa senanensis	Leaves	Antitumoral and phagocytic activity	*In vitro* citotoxicity of human NK cells against K562 cells (leukemia); *in vitro* phagocytic activity and NO production by mouse macrophages; *in vivo* (mouse) antitumoral against S-180 (sarcoma), C38 (lung carcinoma) and Meth-A (sarcoma).	Seki et al. 2008
Sasa senanensis	Leaves	Antioxidant; total phenol and flavonoid content	DPPH, ORAC, Hydroxyl and Superoxide Radical Scavenging Activities, Measurement of plasma ORAC value, Measurement of liver lipid peroxidation, Quantification of reduced glutathione (GSH). Folin Ciocalteu, aluminium chloride.	Khatun et al. 2013
Sasa senanensis	Leaves	Cytotoxicity; anti-UV; anti-HIV; radical scavengin	MTT (HL-60, Normal oral cells, HSC-2, HSC-3, HSC-4, HGF, HPC, HPLF); MTT (HSC-2 cells exposed to UV lamp); HIV infected cells; Superoxide anion (HX-XOD); DPPH.	Matsuta et al. 2011

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Sasa senanensis	Leaves	Antioxidant	DPPH and superoxide radical scavenging.	Matsuta et al. 2009
Sasa senanensis	Leaves	Cell protective effect	Cell protective effect using rat PC12 and human SH-SY5Y neuron model cells from amyloid β-peptide (Aβ)-induced injury. Viability of cells was determined by the MTT method.	Sakagami et al. 2018
Sasa veitchii	Leaves	*In vivo* hepatotoxicity	Hepatotoxicity induced by acetaminophen (APAP) in male ddY mice was used as model. Plasma biochemical analysis, histological analysis, malondialdehyde (MDA) and hepatic GSH levels, total antioxidant capacity, western blot analysis, isolation of total RNA and qRT-PCR assay.	Yoshioka et al. 2017
Sasa veitchii	Leaves	Anti-inflammatory	COX inhibitor screening assay.	Van Hoyweghen et al. 2014
Sasa veitchii	Leaves	Immunomodulatory potential	*In vitro* using the spleen or bone marrow cells of mice. The splenocytes of male DBA/2 and C57BL/6 mice were cultured with bamboo water-soluble methanol precipitation in the presence or absence of PAMPs, and responses were assessed by measuring cytokines.	Sato et al. 2015
Sasa veitchii	Leaves	*In vivo* hepatotoxicity and nephrotoxicity	Hepatotoxicity and nephrotoxicity induced by carbon tetrachloride (CCl_4) in male ddY mice was used as model. Mortality in animal experiments, biochemical analysis, histological analysis, liver Ca concentration determined by atomic absorbance, hepatic metallothionein (MT) protein levels determined by the Cd-saturation/hemolysate (Cd-hem) method, and determination of biological antioxidant power (BAP) in plasma were performed.	Yoshioka et al. 2016
Sasa veitchii	Leaves	Activity against obesity-induced insulin resistance and hepatic steatosis	Obesity induced by a high-fat diet (HF) in male ddY mice was used as model. Plasma biochemical, histopathological, and westerm blot analyses, insulin and oral glucose tolerance tests, and isolation of total RNA and qRT-PCR assay were performed to evaluate activity against on obesity-induced insulin resistance and hepatic steatosis in mice.	Yoshioka et al. 2017
Sasa veitchii	Not specified	Immunochemical cross-reactivity	Enzyme-linked immunosorbent assay.	Sato et al. 2016
Sasa veitchii	Leaves	Antioxidant	DPPH, ABTS, and ORAC.	Van Hoyweghen et al. 2012.

Accepted name (= Synomyn used)	Part of plant	Analyses	Methodology	Reference
Sasa veitchii	Leaves	Antibacterial	Microbroth dilution and scanning electron microscopy of Methicillin-susceptible *Staphylococcus aureus* (MSSA); Methicillin-resistant *Staphylococcus aureus* (MRSA); Vancomycin-sensitive *Enterococcus faecium* (VSE); Vancomycin-resistant *Enterococcus faecalis* (VRE); *S. pneumoniae*; *E. coli*, and *P. aeruginosa*.	Shirotake et al. 2009
Schizostachyum lumampao	Leaves	Total phenol and flavonoid content	Folin-Ciocalteu	Tongco et al. 2014
Schizostachyum zollingeri	Leaves	Antioxidant and antityrosinase	Folin-Ciocalteu, Ferric thyocianate (FTC); Xanthin/Xanthine oxidase superoxide scavenging activity (X/XOD); DPPH; Tyrosinase inhibitory.	Ilham et al. 2008
Shibataea chinensis	Leaves	Total phenol, flavonoid and terpenoid content; and antioxidant	Folin–Ciocalteu; Aluminum nitrate-sodium; Vanillin-glacial acetic acid; DPPH and FRAP.	Ni et al. 2013 (b)

5. Conclusions and future perspectives

One of the main goals of this review was to know which bamboo species have studies reported in the literature. Although bamboo species were used for centuries in traditional China medicine, only 19% of the genera and 5% of total bamboo species were studied in the last 11 years. Furthermore, there is a lack of knowledge regarding the Central and South American bamboo species, mainly for the herbaceous species (Olyreae tribe), with no papers found. Therefore, the largest number of phytochemical studies and biological activities refer to Asian species; this fact makes herbaceous species a potential source of new molecules scaffold to be exploited.

Another issue that guided this research was to understand what is currently known about the composition of secondary metabolites of bamboos. This review showed a lack of knowledge about the chemical composition of bamboo species. Although bamboos are known to have a diversity of flavonoids, in special the *C*-glycosylated ones, there are other classes of constituents also reported in the literature, such as alkaloids and coumarins. Although, few papers used hyphenated techniques to identify these substances.

And finally, addressing the question 'Do bamboos have any medicinal potential?' we observed that there are many bamboo species with their *in vitro* biological potential tested, but the majority of papers reported the antioxidant activity of phenolic compounds, a well-predicted activity for this class of compounds. In comparison with total papers, few of them explored other biological activities for this group of plant, even less the ones using *in vivo* assays.

Perhaps the use of bamboo leaves as antioxidant additives in industrialized foods limited investigations into different traditional uses attributed to bamboos, since the most studied species, *Phyllostachys edulis*, is also one of the species used to prepare the additive known as AOB.

There are few correlations observed between the traditional uses of bamboo species and the bioactivities tested. For example, some studies reported the traditional use for the treatment of mental disorders attributed to human aging, as Alzheimer's disease. However, of all the compiled studies, only one study analyzed the *in vitro* anticholinesterase potential of a single bamboo species.

In the literature, alkaloids are considered a group of substances of great importance for the treatment of neurodegenerative diseases; currently one of the drugs used to treat Alzheimer's disease is Galantamine, an alkaloid derivative. Although the presence of alkaloids has been reported for some bamboo species, these studies are still preliminary.

Moreover, most studies were conducted using extracts, where there is a complex mixture of constituents, which difficults the establishment of a dose-response, or even the elucidation of the bioactive compound and its mechanism of action. Furthermore, a few species were assayed *in vivo* and no bioavailability and pharmacokinetic studies have been reported for them.

Based on what was compiled in this study, it is possible to say that bamboos have economically important metabolites with a possible medicinal application, but it is important to evaluate if the yield of these compounds or extracts is sufficient to make an herbal medicine; few papers discuss or report yields for isolated compounds. In conclusion, further studies on phytochemistry and more *in vivo* assays are still needed to better evaluate the benefits of bamboo to human health.

6. Hypothesis and aim of the present study

For centuries the Asian people, mainly the Chinese, use the leaves of some species of bamboos for the treatment of diverse diseases, mainly diseases of mental disorder that are attributed to the human aging, like Alzheimer for example. Even with the traditional knowledge about the use of Asian bamboos, there are few studies investigating the bamboo role in mental illness.

In this context, Brazil has the greatest diversity of bamboo species in the Americas, with a high degree of endemism, yet nothing is known about the medicinal potential of these species. Therefore, considering that Asian species are used in tradicional medicine and have biological activities attributed to the presence of phenolic substances, the hypotheses of this study is that the secondary metabolites, especially phenolic compounds, present in Brazilian bamboos have biological potential against diseases that affect cognition and memory.

This study aimed to analyze the potential of Brazilian bamboo extracts in the inhibition of two classes of cholinesterase enzymes, and analyze the antioxidant potential of these extracts. Besides to analyze the toxicological effect of the aqueous extracts on the morphology, locomotion, and behaviour of zebrafish larvae; and also to analyze the potential of these extracts in improving the cognitive and memory functions in adults of zebrafish (*Danio rerio*).

7. Studied species

Six species of Brazilian bamboo were chosen for this study, four of them belonging to the Bambuseae tribe (tropical woody bamboo): *Aulonemia aristulata* (Döll) McClure; *Filgueirasia arenicola* (McClure) Guala; *Filgueirasia cannavieira* (Silveira) Guala; and *Merostachys*

pluriflora Munro ex. C. G. Camus. Besides two species from Olyreae tribe (herbaceous bamboo): *Olyra glaberrima* Raddi and *Parodiolyra micrantha* (Kunth) Davidse & Zuloaga (Figure 10).

Aulonemia is a genus with about 50 described species, distributed from Mexico to southern Brazil, occurring in high elevation forests of the Andes, Guiana Shield, Central Brazil and in the Atlantic rainforest (Viana and Filgueiras 2014). *Aulonemia* species have different habit forms, which are intrinsically related to the environment where they are found. The majority of the species are forest of scandant or supportive habit (Viana 2010). In Brazil there are 16 species of *Aulonemia*, 15 of which are endemic (Greco et al. 2015).

Aulonemia aristulata is an endemic species (Grombone-Guaratini et al. 2011) the culms are initially erect, becoming climbing over forest vegetation, and tend to form dense agglomerates in forest clearings or understory propagating through amphipodial rhizomes (Viana 2010).

Aulonemia aristulata is a typically forest species, but it occasionally grows on the banks of streams, in open places. In the Atlantic Forest domains, it is relatively common in forest remnants at intermediate altitudes, between 800 and 1400 m, both in seasonal forest and in rain forest (Viana 2010), and there are records of its occurrence in the northeast (BA), central-west (GO, DF), southeast (MG, ES, SP) and south (PR, SC) of the country (Greco et al. 2015).

Filgueirasia has only two species and is an endemic genus of the Brazilian Cerrado, the period between mass flowerings is unknown although it is not less than eleven years and is probably more than twenty, and flowering may also be linked to burning (Guala 2003).

Filgueirasia arenicola occurs in the northeast (BA), central-west (MT, GO, MS) and southeast (MG) regions; *Filgueirasia cannavieira* occurs only in the central-west (GO, DF) and southeast (MG) regions (Greco et al. 2015). They are both good forage and are eaten by both domestic stock and wildlife (Guala 2003).

Merostachys is widely distributed in Central and South America (Sedulsky 1992), Brazil being the country with the highest diversity of species, with 53 species, of which 50 are endemic (Filgueiras and Gonçalves 2004).

Merostachys pluriflora is an endemic bamboo from the Atlantic Rain Forest, Brazil (Filgueiras and Gonçalves 2004). *M. pluriflora* possesses peculiar morphological characteristics; their culms can measure 7–12 m, erect on the base and pending at the apex, and may have 5–9 mm in diameter; a very striking feature of this species is the presence of dense white trichomes above and below the nodal line (Shirasuna and Filgueiras 2013). There are records of the occurrence of this species in the southeast (SP, RJ) and south (SC) regions in Brazil, and his flowering cycle is not yet known (Shirasuna and Filgueiras 2013, Greco et al. 2015).

On the herbaceous bamboos, *Olyra* is the genus with the largest number of native species contributing to 7.8% of the diversity (Filgueiras and Gonçalves 2004). *Olyra* and other herbaceous bamboos usually flower each year for longer or shorter periods of time (Soderstrom and Zuloaga 1989).

In Brazil, 20 species of *Olyra* have already been reported, of which 6 are endemic. *Olyra glaberrima* occurs in the northeast (BA, PE), southeast (ES, SP, RJ) and south (SC) regions (Greco et al. 2015). This species measures between 0.5–1 m high, erect, with pachymorphic rhizome and short neck, and its culms are not branched (Greco and Zannin 2017).

Parodiolyra is a genus with a small number of species (5 ssp) (Jesus Junior et al. 2012), four of which were segregated from *Olyra* (Soderstrom and Zuloaga 1989). *Parodiolyra* species are perennial, growing in erect clumps, sometimes slightly decumbent or recurved, with rarely branched culms and symmetrical, lanceolate to broadly oval leaf blades (Jesus Junior et al. 2012).

Parodiolyra micrantha has a wide distribution in the country; it is easily found in several Brazilian states, from the north (RR, PA, AM, AC) to south (PR, SC, RS). They are plants that measure between 1–4 m high, erect at the base and arched at the apex, its culms are presented with or without branches (Greco and Zannin 2017).

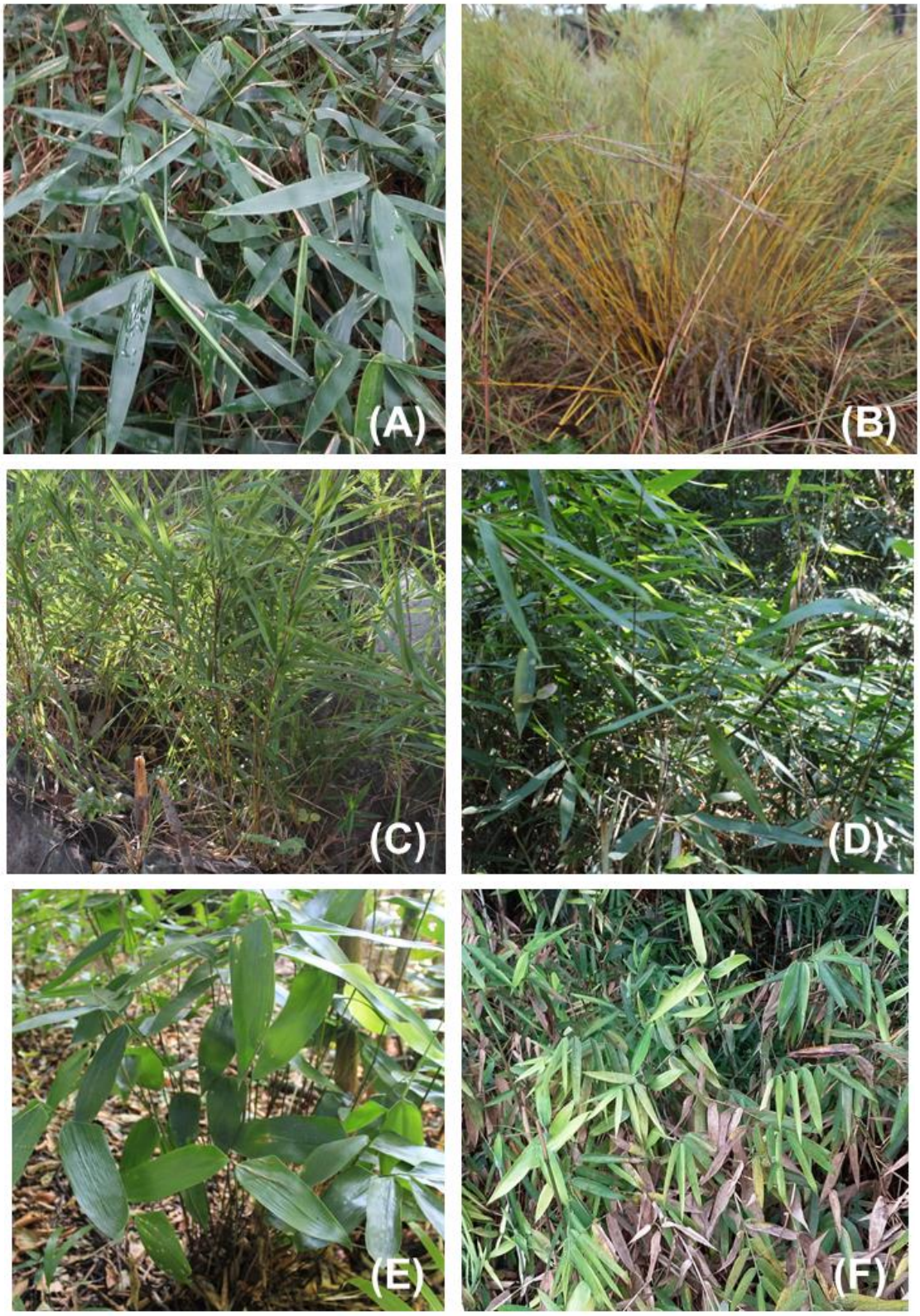


Figure 10 - Brazilian bamboo species. **(A)** *Aulonemia aristulata*; **(B)** *Filgueirasia arenicola;* **(C)** *Filgueirasia cannavieira;* **(D)** *Merostachys pluriflora;* **(E)** *Olyra glaberrima;* **(F)** *Parodiolyra micranta.* Photos: (**A,D,E, F**) – Gagliano J and Furlan CM ; (**B,C**) – Grombone-Guaratini, MT.

8. Plant collection and sample preparation

Leaves from *Aulonemia*, *Merostachys*, and *Parodiolyra* were collected in São Paulo at Parque Nacional das Fontes do Ipiranga (PEFI), located at the Instituto de Botânica de São Paulo (IBt) (Figure 11) (authorization number CNPq 010006/2015-0). *Filgueirasia* species were collected in Goias, Brazil and these species were given by Drª Maria Tereza Grombone-Guaratini, from IBt. All these bamboo species were indentified by Dr. Tarciso Filgueiras (*in memoriam*).

Olyra glaberrima was collected in the forest reserve of the University City "Armando Salles de Oliveira" (CUASO) and identified by Dr. Milton Groppo (Figure 11; Table 4).

Plant material was dried in an oven at 40°C and powdered. Dried and powdered leaves were subjected to different types of extractions, according to applied methodology described forward in *Chapter* **1** and **2**.

Table 4 – Plant collection data.

Species	City	State	Herbarium	Voucher	Collection date
Olyra glaberrima	São Paulo	SP	SPF	Gagliano 03	05/03/2017
Parodiolyra micranta	São Paulo	SP	SPF	Gagliano 02	10/21/2016
Aulonemia aristulata	São Paulo	SP	SP	SP398161	10/21/2016
Filgueirasia arenicola	Mineiros	GO	SP	SP326929	06/17/2015
Filgueirasia cannavieira	Cavalcante	GO	SP	SP248877	06/13/2015
Merostachys pluriflora	São Paulo	SP	SPF	SPF221335	06/30/2014

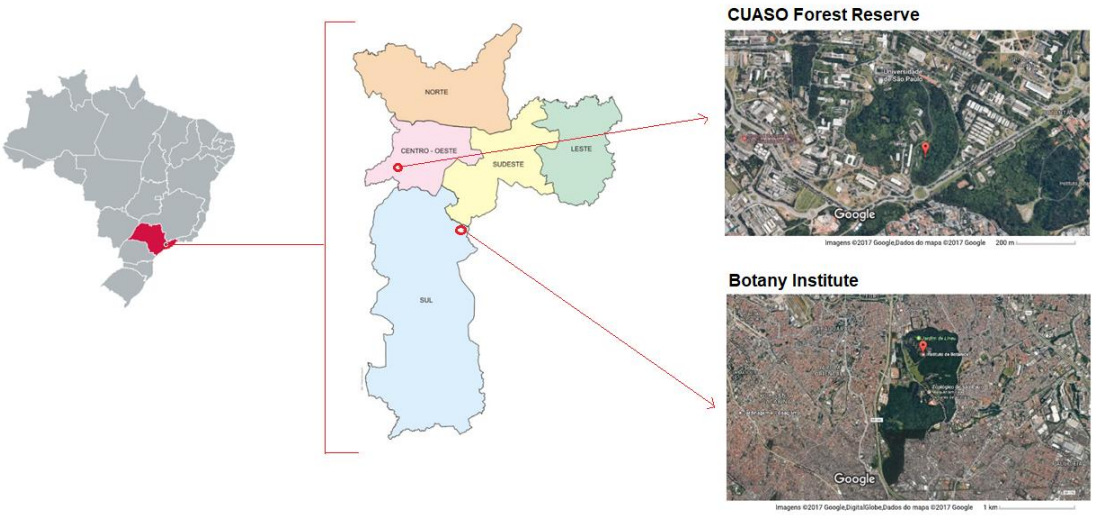


Figure 11 – Location of collection sites at São Paulo. Source: edited by Google images.

Chapter 1

Chemical characterization of six Brazilian bamboo species

1.1. Introduction

Brazil has the greatest diversity of bamboo species in the Americas. Amazon and Atlantic Rain forests are the main centers of diversity for this group of plants. Moreover, some species occur in the Cerrado (Brazilian savannah), in high altitude grasslands, and in rocky fields (Greco et al. 2015).

Among the 256 species found in Brazil, 176 are endemic. *Merostachys* (43 species) and *Chusquea* (45 species) are the most common genera of Bambuseae, and also, they have the highest endemism of species, 41 and 42, respectively. For Olyreae, *Pariana* (29 species) and *Olyra* (20 species) are the richest genera (Greco et al. 2015).

Brazil is one of the countries which show the greater diversity of the native herbaceous bamboos (tribe Olyreae) in the world with 93 species. Olyreae species represent 36.1% of the native bamboos in the country (Greco et al. 2015).

Although Brazil possesses the greatest diversity of native species, several exotic bamboo species were introduced during the period of colonization; these species are widely distributed around the country and have a large number of uses (Londoño 1998). The native bamboos are very poorly known by the Brazilian people; only exotic species are cultivated as ornamental, or for making handcrafts and for uses in small rural constructions (Greco et al. 2015).

For Greco et al. (2015), the best examples of success in bamboo exploitation in Brazil is in energetic and paper pulp utilization in the states of Maranhão and Pernambuco, Northeast of the country. In these Brazilian states, two companies have developed a cultivated area of 35 thousand hectares of *Bambusa vulgaris* Schrad., that are used to manufacture of duplex cards.

Besides that, São Paulo has a large production of *Phyllostachys edulis* (Carrière) J. Houz., *Phyllostachys aurea* Carrière ex Rivière & C. Rivière and *Dendrocalamus asper* (Schult. & Schult. f.) Baker ex K. Heyne, these bamboo species are cultivated and partially used for shoot production (Greco et al. 2015).

However, these species are exotic, lacking studies that evaluate the potential of application of the Brazilian bamboos in the most varied industrial segments, especially for drug discovery.

As seen previously, in the last 11 years only 5% of the bamboo species has been studied phytochemically; phenolic compounds, especially flavonoids, were the most secondary metabolite class reported as major constituents of bamboo species.

Some flavonoids are useful in the chemotaxonomy of monocots, attributing taxonomic importance to the differences among the levels of oxidation of these molecules, as well as to the types and nature of substitutions in the main molecule structures (Harborne and Williams 1976).

In the study by Harborne and Williams (1976), flavones as tricin and *C*-glycosides were found to be the major flavonoids in 93% of the Poaceae samples (274 species from 121 genera). Therefore, these authors concluded that the flavonols kaempferol and quercetin are rare in Poaceae, as well as the flavones apigenin and luteolin *O*-glycosides. On the other hand, Tricin is almost universal in Poaceae.

Therefore, taking into account what is known about the chemotaxonomy of grasses, and the lack of phytochemical studies on Brazilian bamboo species, one of the hypotheses of this work is that Brazilian bamboos, as the Asian bamboo species, will also present a great diversity of flavones, especially the *C*-glycosides, but also other groups of secondary metabolites, like chromenes, alkaloids, and terpenoids.

From 2007 to 2018 only 10 phytochemical studies using Brazilian bamboo species were published, and only three genera were investigated, *Aulonemia*, *Merostachys*, and *Guadua*, all of them belonging to Bambuseae. Therefore, considering that Brazil has a great diversity of bamboos, and many of them are endemic, together with a huge lack on their chemical composition, this chapter aimed to chemical characterize six species of Brazilian bamboo, using different chromatographic techniques for separation and compound identification.

1.2. Material and methods

1.2.1. Plant material and sample preparation

This study used six Brazilian bamboo species: *Aulonemia aristulata* **(AA)**, *Filgueirasia arenicola* **(FA)**, *Filgueirasia cannavieira* **(FC)**, *Merostachys pluriflora* **(MP)**, *Olyra glaberrima* **(OG)**, and *Parodiolyra micrantha* **(PM)**.

Leaves of each species were dried in an oven at 40°C and powdered. After that, the powdered leaves were submitted to two different extraction methods.

1.2.1.1. Maceration

Dried and powdered leaves (200 g) were subjected to successive maceration, first with hexane during 15 days; after that, the same plant material was subject to maceration with 70% ethanol for more 15 days. For both solvents, the maceration was in the dark, at room temperature, and with solvent exchange every two days.

1.2.1.2. Infusion

The infusion consisted of immediate immersion of about 7 g of dried and powdered leaves of the studied plant species in 500 mL of sterile, freshly boiled distilled water, leaving them in the extraction for a period of 20 minutes.

Hexane and hydroethanol extracts from maceration, and aqueous extracts obtained by infusion were concentrated under reduced pressure below 50℃ using a rotary evaporator, and freeze-dried to obtain their yields. These extracts were used for HPLC and GC analyses described below and for all bioassays described forward in *Chapter* 2.

1.2.2. Determination of plant extract yield

The yield of freeze-dried extracts based on dry weight was calculated using the following equation:

$$\text{Yield } \% = (W1 \times 100) / W2$$

Where W1 is the weight of the freeze-dried extract and W2 is the weight of the dry plant material used.

1.2.3. Colorimetric assays

To quantify total phenolic and flavonoid content 40 mg of the dried-powdered bamboo leaves were submitted to extraction for 1 h using 80% methanol at 70°C in a dry bath. The extracts were filtered, and the volume was adjusted to 10 mL.

For these analyses, all samples, negative and positive controls were analyzed in triplicate. The levels were determined using a microplate reader, Synergy H^1 equipment (BioTek, Inc.).

1.2.3.1. Total phenol content

The total phenolic content was determined according to the Folin–Ciocalteu method modified by Furlan et al. (2015). 190 µL of ultrapure water, 10 µL of Folin–Ciocalteu reagent, 50 µL of 80% methanol extracts of bamboo leaves (80% methanol was used as negative control), and 50 µL of 10% sodium carbonate was added to each well of a 96-well microplate. The material was homogenized, and the plate was incubated for 30 min at 40°C. The total phenol content was measured at 760 nm. The results were compared to a standard curve of Gallic acid (5–80 µg/mL) and were expressed as milligram of gallic acid equivalent per g of plant material (mg/g GAE).

1.2.3.2. Total flavonoid content

The flavonoid content was determined according to the aluminum chloride method which was modified from Santos and Furlan (2013). 100 µL of each bamboo extracts were added to 96-

well microplate with 100 µL of 5% aluminum chloride (80% methanol was used as negative control). The material was homogenized, and the flavonoid content was measured at 420 nm. The results were compared to a standard curve of Quercetin (3.6–84 µg/mL) and were expressed as milligram of quercetin equivalent per g of plant material (mg/g QE).

1.2.4. GC-MS analysis

For GC-MS analysis, compound identification was done by comparing its retention time, Linear Retention Index (LRI), and mass fragmentation pattern to commercial standards and literature data.

Experimental data were compared with NIST digital library spectra (v2.0, 2008), European Mass Bank database (http://massbank.eu/MassBank/Index), Golm Metabolome Database (GMD) (http://gmd.mpimp-golm.mpg.de/), Scifinder (https://scifinder.cas.org/), NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/), and the available literature.

For each substance, the linear retention index (LRI) was calculated using an *n*-alkane standard mixture (C8-C20 from Sigma-Aldrich and C19-C40, except the C27 homolog, from PolyScience). The LRI was calculated according to the following equation (Viegas and Bassoli 2007): $LRI = 100*[(RT_c-RT_n)/(RT_{n+1}-RT_n) + C_n]$. Where RT_c – retention time of compound; RT_n – retention time of the previous *n*-alkane; RT_{n+1} – retention time of the posterior *n*-alkane; Cn - number of carbons from the previous *n*-alkane.

1.2.4.1. Polar compounds

Dried and powdered bamboo leaves (50 mg) were transferred to 2 mL microtubes and added 1400 µL of MeOH (pre-cooled to -20°C), 60 µL of the internal standard Ribitol (0.2 mg/mL in ultrapure water), and the solution was vortexed for 10s. Then, the samples were shaken at 950 rpm in thermo mixer for 10 min at 70°C and centrifuged at 11,000g for 10 min. After that, the supernatants were transferred to glass tubes and added 750 µL of $CHCl_3$ (pre-cooled to -20°C) and 1500 µL of H_2O (pre-cooled to 4°C); samples were mixed for 10s and centrifuged at 2,200g for 15min (according to Nagai 2017, with some modifications).

Lastly, an aliquot of 100 µL from the upper phase (polar) was taken and transferred straight to inserts and dried in a vacuum concentrator without heating and subsequently subjected to the derivatization reaction.

For the derivatization reaction were used Methoxyamine hydrochloride (CAS 593-56-6, Sigma) in pure Pyridine (20 mg/mL) and N-Methyl-N-(trimethylsilyl) trifluoroacetamide silylation reagent (MSTFA, CAS 24589-78-4). For this reaction, it was added 28 µL of

methoxyamine hydrochloride in each aliquot of each sample and kept at 37°C for 2h. After that, 48 µL of MSTFA was added and kept at 37°C for more 30 min.

For GC-EIMS analysis (6850 Network GC System Agilent-Agilent 5975 VL MSD), 1 µL of each sample was injected in splitless mode. It was used a capillary column VF 5MS (30 m, 250 µm, 0.25 µm) and a pre-column (10 m, 0.25 mm), with helium constant flow at 1 mL/min. The injector temperature was 230°C. The programming was: 0-5 min isothermal at 70°C; ramp 5°C/min to 330°C and kept for 5 min. Mass spectrometer parameters were: 70 eV electron multiplier voltage; ion source at 200°C; quadrupole temperature to 150ºC; mass spectrum at 2 scans/s with 50 to 600 m/z scan. Solvent delay: 9-10 min.

For substance analysis, the following criteria were adopted: threshold of 20 and only substances corresponding to 1% of the total area were considered.

1.2.4.2. Nonpolar compounds

Crude hexane extracts were solubilized in hexane (1 mg/mL), dried under vacuum, and derivatized. Derivatization process consisted of dissolving previously dried aliquot of the hexane extract in 25 µL of pyridine and 25 µL of BSTFA (*N*, *O*-bis-(trimethylsilyl)-trifluoroacetamide, Sigma-Aldrich), heated at 70ºC for 1 hour.

After derivatization process, 1 µL of each sample was injected in spitless mode and analyzed by GC-EIMS (6850 Network GC System Agilent-Agilent 5975 VL MSD) equipped with a HP-5MS capillary column (5% phenyl, 95% polydimethylsiloxane – Agilent, length 30 m, ID 250 µm, 0.25 µm film thickness), with helium as carrier gas (1 mL min^{-1}). During this analysis, the injector and detector temperatures were 250°C and 350°C, respectively. The column temperature program was the following: 150°C for 5 min, raising 5°C min-1 till 325°C. The analysis employed an ionization voltage of 70 eV and an ion source temperature of 230°C.

For substance analysis, the following criteria were adopted: threshold of 15 and only substances corresponding to 1% of the total area were considered.

1.2.5. HPLC-ESIMS/MS analysis

For this analysis, all hydroethanol and aqueous extracts were solubilized in 80% Methanol (5 mg/mL), filtered (PTFE 0.45 µm) and submitted by HPLC-ESIMS/MS. The liquid chromatograph (Shimadzu, Kyoto, Japan) was equipped with a controller (CBM-20A), two pumps (model LC-20AD), an automatic injector (SIL-20AHT), a column oven (CTO-20A), and an UV/Vis detector (SPD-20A). Chromatographic separation was done using a Kinetex C-18 column (Phenomenex, 100 A, 100 x 1 mm, 2.6 µm PFP) at 40ºC, with a solvent flow rate of 0.2 mL/min infused directly into the mass spectrometer, and 5 µL of injection volume.

The mobile phase consisted of 0.1% formic acid and acidified acetonitrile (0.1% acid formic, acidified-ACN) starting with 0% of acidified-ACN at 0 min, increasing to 10% (0-10 min), isocratic for 10 min (10-20 min), raising from 10% to 15% (20-25 min), isocratic for 10 min (25-35 min), ranging from 15% to 30% (35-50 min), increasing from 30% to 45% (50-71 min), reaching 100% at 90 min, and isocratic for 2 min. Separated compounds were monitored at 340 nm.

The mass spectrometer (BrukerMicrOTOF-QII) operated in positive and negative mode, and nitrogen was used as a nebulizer (4 Bar) and dried gas (flow of 8 L/min). The capillary voltage was set at 4,500 V and drying temperature to 200ºC. The collision and the quadrupole energy were set to 12 and 6 eV, respectively. RF1 and RF2 funnels were programmed to 400 and 200 Vpp, respectively. The monitored mass range was 100-1000 kDa. MS was previously calibrated using sodium formate.

This analysis was performed at the Department of Fundamental Chemistry, Chemistry Institute, University of São Paulo, under the supervision of Ph.D Lydia F. Yamaguchi and Ph.D Massuo Jorge Kato.

All raw data files obtained from the HPLC analysis of the extracts were converted to .mzXML using Data Analysis 4.3 software (Bruker) in order to transform spectra from profile to centroid mode. The .mzXML files were uploaded on Global Natural Product Social Molecular Networking (GNPS) through WinSCP (version 5.17.1) and analyzed with the GNPS platform (http://gnps.ucsd.edu). For the spectral library search, precursor ion mass tolerance was 2.0 Da and MS/MS fragment ion mass tolerance was set at 0.5 Da. In this work, only substances that had a cosine index equal to and/or greater than 0.70 were integrated.

1.2.6. Heat map, PCA and Hierarchical analysis

GraphPad Prism version 8.0.2 program was used to build the heat maps. For the principal component analysis (PCA) and Hierarchical analysis was used the PAST version 4.03 program.

1.3. Results and discussion

1.3.1. Extraction yielded

This study used six Brazilian bamboo species: *Aulonemia aristulata* (AA), *Filgueirasia arenicola* (FA), *Filgueirasia cannavieira* (FC), *Merostachys pluriflora* (MP), *Olyra glaberrima* (OG), and *Parodiolyra micrantha* (PM). Amongst the six species, PM and OG showed the highest yields for both extraction methods, maceration and infusion, between 13% and 20%. On the other hand, FA had the lowest yields for maceration (5%) and infusion (3%) (Figure 1.1).

In general, the extracts with the highest yields were those obtained using polar solvents, such as hydroethanol and aqueous extracts. These results can infer higher amounts of polar substances in relation to nonpolar as major constituents in leaves from all studied species.

PM and OG, belonging to Olyreae, and these species were the ones with the highest extract yields. However, there are no reports in the literature regarding phytochemical studies using herbaceous bamboo species.

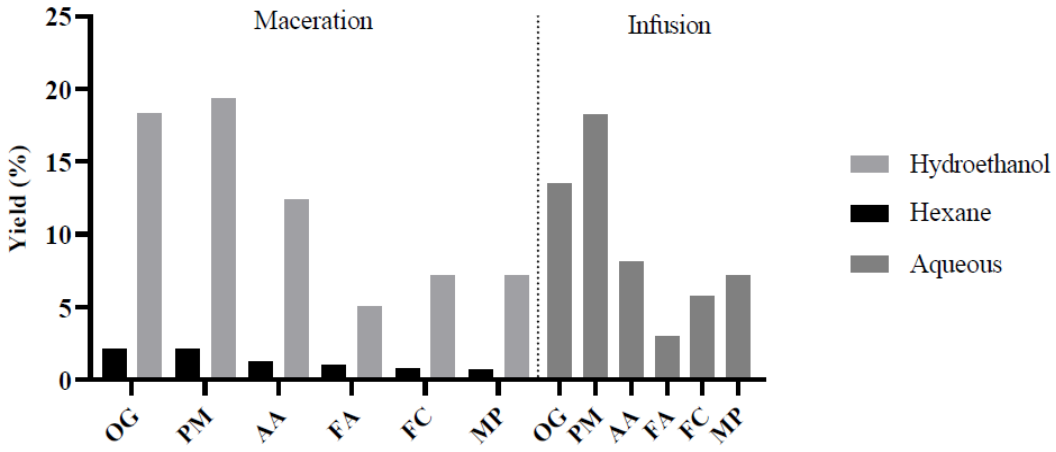


Figure 1.1 – Percentage of yield of lyophilized extracts produced by maceration and infusion. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.*

Wroblewska et al. (2019) reported the yield of hydroethanol of *Merostachys pluriflora* and *Aulonemia aristulata* leaves of 13.9% and 10.1%, respectively. In the present study, *A. aristulata* (AA) showed similar yield (12%) but *M. pluriflora* (MP) presented lower extract yield (7%), when comparing both studies. However, comparing with a previous study (Gagliano et al. 2018), *M. pluriflora* showed 7.7% of hydroethanol extract yield, corroborating the result observed in the present study.

Extraction methods are directly related to extract's yield. Wroblewska et al. (2019) used 60% ethanol, and the Soxhlet apparatus for extraction until exhaustion which may enhanced extract's yield; while in the present study was applied successive maceration in hexane and 70% ethanol.

Moreira (2019) reported a 12% yield of hydroethanol extract from leaves of *Merostachys neesii*. In this case, the author used the same extraction method as well as the same proportion of ethanol used in the present study. So, it is possible to infer that *M. neesii* showed high yield when compared to *M. pluriflora* (7%), a species belonging to the same genus.

1.3.2. Total phenolic and flavonoid contents

The highest levels of total phenolic and flavonoids were observed for PM. For this species, the total phenolic content was almost three times higher than that observed for the other species. Contents of total phenolic (mg/g GAE) among the six analyzed species followed PM>FA>AA>FC>OG>MP, while for flavonoid content (mg/g QE) the species followed PM>OG>AA>MP>FC>FA (Figure 2.2).

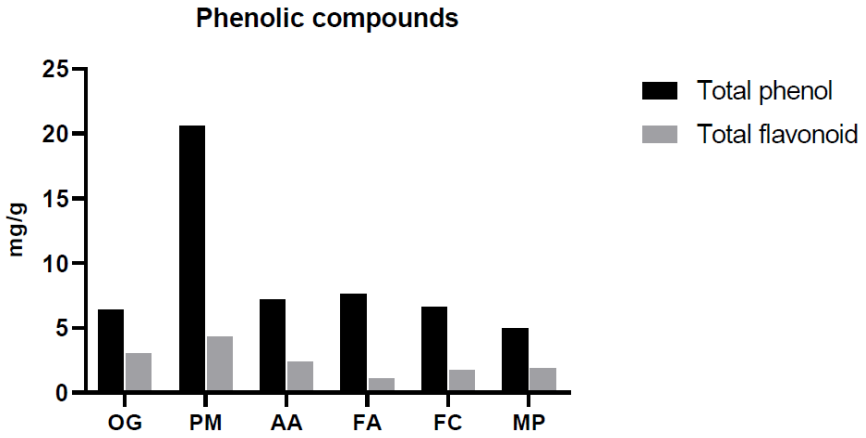


Figure 1.2 – Total phenolic and flavonoid contents in leaves of: **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.* Total phenol (mg/g GAE). Total flavonoid (mg/g QE). (GAE): Gallic acid equivalent; (QE): Quercetin equivalent.

As observed in Figure 1.2, PM, FA, and FC showed low levels of flavonoids, being possible to infer that a large part of the phenolic compounds in these species corresponds to other phenolic groups, but not to flavonoids. OG, AA, and MP showed almost half of the phenolic compound contents as flavonoids.

Folin-Ciocalteu assay is the most commonly used procedure to determine total phenolic compounds in plant extracts. However, this method is not just specific for total phenolic determinations, because it is known that other types of compounds that may be present in high abundance in plant extracts, as reducing sugars and amino acids can also reduce the Folin reagent, whether or not overestimating the levels of phenolic compounds in the sample (Sanchez-Rangel et al. 2013).

As the Folin-Ciocalteu protocol has limitations to estimate the content of total phenolic compounds, the colorimetric method that uses aluminum chloride to estimate total flavonoids also has its limitations (Papoti et al. 2011).

Due to their structure, flavonoids can easily chelate metal ions forming complexes metal-flavonoid. It seems that metal-flavonoid complexation reactions are particularly appropriate for analytical objectives as formed complexes bear exceptional spectrophotometric characteristics. Aluminium-complexation reaction in the presence of $NaNO_2$ seems to be nonselective for flavonoid assessment as the codetermination of o-diphenols strongly affects the results. Besides that, the reaction environment (pH, solvent) also influences analytical data (Papoti et al. 2011).

Although the colorimetric methods are widely used as a first approach in the phytochemical studies of plant extracts, due to their characteristics as less time for analysis, low cost, and for being easy to reproduce.

Several studies still use colorimetric assays to estimate total phenolic compounds and flavonoid content in bamboo extracts (see *Introduction*, Table 2, page 13). So, it was possible to infer that sophisticated analytical techniques that use equipment for separation and detection of compounds are not yet widely accessible for many research laboratories.

1.3.3. GC-MS analysis

GC-MS analyzes revealed 102 peaks, being 66 of then identified. From hot methanol extracts (polar extracts) it was identified 40 constituents (Table 1.1) and from hexane extracts (nonpolar extracts) 26 constituents were identified (Table 1.2). For both extracts, constituents were estimated by their relative percentage (%).

Polar extracts showed constituents as soluble sugars (monosaccharide, disaccharide, and sugar alcohol), amino acids and derivatives, organic acids, and phenolic acids (Figure 1.3).

It was detected nine amino acids: Norvaline, Valine, Serine, Isoleucine, Threonine, Proline, and Aspartic acid; and two amino acid derivatives: 5-oxoproline and gamma-Aminobutyric acid (GABA). Contents of amino acids and derivatives ranged from 1.9% to 18.5%. Asparagine, 5-oxoproline, and GABA were the amino acids detected in all of the six bamboo species. Serine was the most abundant amino acid, especially in AA (5.3%).

Four monosaccharides, Fructose, Glucose, Galactose, and a Methyl galactoside, and one disaccharide, Sucrose, were detected. For sugar alcohol was observed Glycerol, Erythritol, Sorbitol, and *Myo*-Inositol. Soluble sugars in all species corresponded to at least half of the constituents identified in polar extracts (Table 1.1).

Among the sugar acids, Glyceric acid was observed in higher levels in OG, FA, FC, and MP, while Threonic acid was the major sugar acid in PM and AA. Sorbitol was the most abundant sugar alcohol in OG, PM, AA, FA, while Erythritol was the major polyol in FC and MP. For organic acids, the most abundant constituent was Malic acid for OG, AA, FA, FC; Mesaconic and Aconitic acids were the major organic acids in PM and MP, respectively.

Table 1.1 – Relative percentage of constituents detected by GC-EIMS in all bamboo polar extracts. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.*

Peak	R.T (min)	Constituent	Relative percentage (%)						Match NIST02	LRI	LRI GMD
			OG	PM	AA	FA	FC	MP			
1	14.9	Norvaline (2TMS)	0.2	0.3	1.2	0.0	0.0	0.6	811	1212.19	1229.91
2	15.0	Valine (2TMS)	0.1	0.0	0.0	0.0	0.0	0.0	801	1214.54	1207.10
3	15.8	Urea (2TMS)	0.1	0.0	0.3	0.4	0.2	0.0	819	1245.55	1234.56
4	16.2	Serine (2TMS)	1.4	0.7	5.3	0.6	0.0	0.4	919	1257.23	1254.21
5	16.3	Ethanolamine (3TMS)	1.5	0.7	3.3	8.2	5.6	6.0	939	1261.67	1259.94
6	16.6	Glycerol (3TMS)	2.7	3.2	9.2	13.6	10.4	4.3	905	1271.88	1262.29
7	17.1	Isoleucine (2TMS)	0.1	0.1	0.4	0.0	0.0	0.2	803	1290.09	1286.54
8	17.2	Threonine (2TMS)	0.6	0.5	2.9	0.4	0.0	0.3	864	1292.84	1290.45
9	17.2	Proline (2TMS)	0.1	0.0	0.0	0.0	0.0	0.0	788	1295.05	1295.77
10	17.5	Maleic acid (2TMS)	0.2	0.1	0.0	0.4	0.3	0.2	830	1303.42	1300.72
11	17.8	Succinic acid (TMS)	1.0	0.1	0.8	1.1	1.1	0.5	924	1314.32	1310.65
12	18.1	Glyceric acid (3TMS)	0.7	0.5	1.9	1.2	2.0	3.3	893	1327.57	1319.94
13	18.5	Itaconic acid (2TMS)	0.5	0.3	0.5	0.6	0.2	0.8	850	1342.25	1339.09
14	18.7	Fumaric acid (2TMS)	0.2	0.1	0.4	0.7	3.7	0.5	815	1350.92	1346.94
15	20.0	Mesaconic acid (2TMS)	0.1	2.6	0.1	0.3	0.1	0.3	800	1399.92	*
16	20.6	Aspartic acid (2TMS)	0.6	0.1	0.9	0.9	0.1	0.4	857	1423.67	1422.39
17	22.2	Malic acid (3TMS)	9.2	1.4	9.8	7.8	8.7	2.6	913	1484.53	1479.34
18	22.6	Erythritol (4TMS)	0.3	0.3	0.9	5.9	17.8	3.9	826	1502.18	1457.51
19	22.7	Salicylic acid (2TMS)	0.3	0.2	0.7	0.4	0.0	0.5	874	1505.87	1506.80
20	23.0	5-oxoproline (2TMS)	0.6	0.5	1.1	2.4	0.3	1.4	852	1519.62	1521.73
21	23.2	GABA (3TMS)	0.6	0.6	3.7	3.2	1.1	2.4	863	1525.24	1527.46
22	23.5	Threonic acid (4TMS)	0.4	0.9	2.5	1.3	0.7	3.0	848	1537.84	1549.65
23	23.7	Phenylalanine (TMS)	0.2	0.1	1.1	0.7	0.0	0.2	829	1547.76	1556.05
24	24.9	Asparagine (2TMS)	1.2	0.1	1.9	0.5	0.4	0.3	854	1597.42	1599.53
25	26.1	3.4.5-Trihydroxypentanoic acid (4TMS)	0.1	0.0	0.1	0.0	0.5	0.2	863	1652.80	*

Peak	R.T (min)	Constituent	Relative percentage (%)						Match NIST02	LRI	LRI GMD
			OG	PM	AA	FA	FC	MP			
26	27.9	NI-1	0.2	0.4	1.8	0.7	2.3	5.7	-	1731.18	*
27	28.2	Aconitic acid (3TMS)	0.3	0.8	0.3	0.3	0.1	3.2	717	1746.61	*
28	28.4	Ribonic acid (5TMS)	0.2	0.2	0.4	0.8	0.4	0.8	848	1754.87	1751.51
29	28.8	NI-2	0.1	0.2	0.4	0.9	0.4	0.6	-	1771.86	*
30	29.3	Azelaic acid (2TMS)	0.1	0.1	0.2	0.6	0.4	0.6	779	1793.98	*
31	29.6	Citric acid (4TMS)	1.2	0.7	1.9	1.7	1.0	3.0	853	1811.87	1803.92
32	30.4	NI-3	0.5	0.9	1.1	1.6	9.5	0.4	-	1847.73	*
33	30.6	Fructose, *O*-methyloxime (5TMS)	29.7	43.1	22.9	11.1	10.7	26.0	922	1861.02	1853.93
34	31.1	Glucose, *O*-methyloxime (5TMS)	21.9	30.4	14.1	12.6	10.6	15.6	940	1885.40	1880.50
35	31.4	Galactose, *O*-methyloxime (5TMS)	3.7	6.2	2.6	2.3	1.9	2.9	880	1900.36	1902.42
36	31.7	Sorbitol (TMS)	0.6	1.8	1.2	10.0	4.4	3.0	875	1916.56	*
37	32.2	*p*-Coumaric acid (TMS)	0.0	0.1	0.3	1.6	0.6	0.7	792	1938.57	*
38	32.4	Gallic acid (4TMS)	0.6	0.4	1.2	2.6	2.0	2.5	846	1949.72	*
39	33.1	Gluconic acid (6TMS)	0.2	0.2	0.1	1.7	0.5	0.4	810	1986.73	1907.02
40	33.3	(3-Hydroxy-4-methoxyphenyl)ethylene glycol (3TMS)	0.1	0.0	0.4	0.5	1.1	0.7	800	1997.24	*
41	34.8	*Myo*-Inositol (6TMS)	0.3	0.3	0.8	0.4	0.2	0.9	907	2077.19	2080.20
42	38.8	Methyl galactoside (4TMS)	0.0	0.1	0.3	0.0	0.0	0.1	807	2298.69	*
43	43.8	Sucrose (8TMS)	17.3	0.6	0.6	0.2	0.8	0.3	890	2599.80	2623.04
		Amino acid and derivatives:	**5.5**	**3.0**	**18.5**	**8.7**	**1.9**	**6.3**			
		Soluble sugars:	**76.7**	**86.1**	**52.8**	**56.1**	**56.7**	**57.1**			
		Organic acids:	**14.4**	**8.0**	**19.6**	**18.8**	**20.7**	**20.0**			
		Organic compounds:	**1.6**	**0.8**	**3.6**	**8.7**	**5.9**	**6.0**			
		Phenolic acids:	**0.9**	**0.7**	**2.2**	**4.6**	**2.6**	**3.7**			
		Unknown:	**0.9**	**1.5**	**3.3**	**3.2**	**12.2**	**6.8**			

-: data with any hit in the used library ***:** values not available

LRI: Experimental Linear Retention Index. **LRI GMD:** Linear Retention Index available in Golm Metabolome Database.

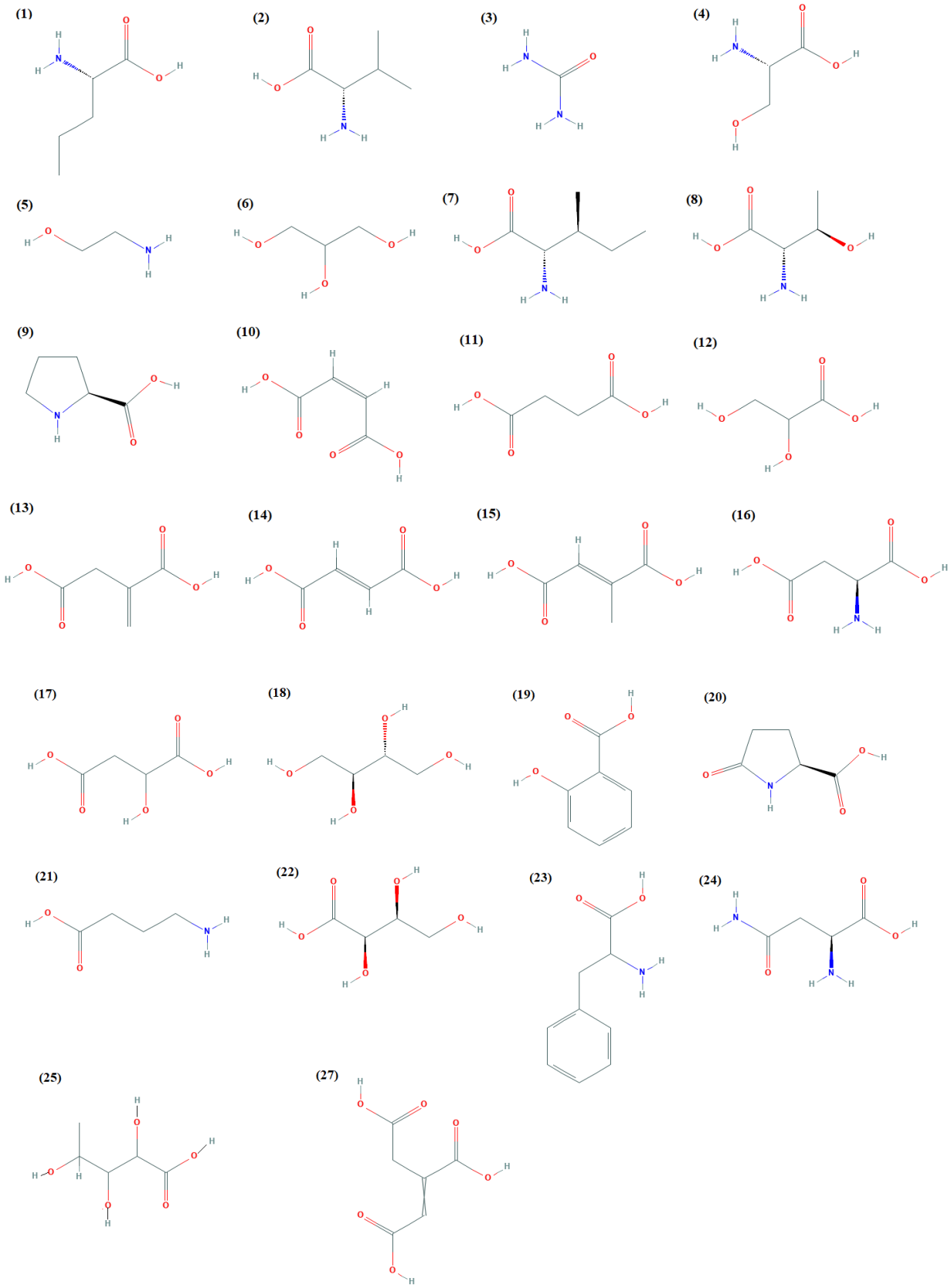


Figure 1.3 – Compounds identified by GC-EIMS in the polar extracts. The numbers above each chemical structure correspond to those shown in Table 1.1. Chemicals structures source: Pubchem, 2020.

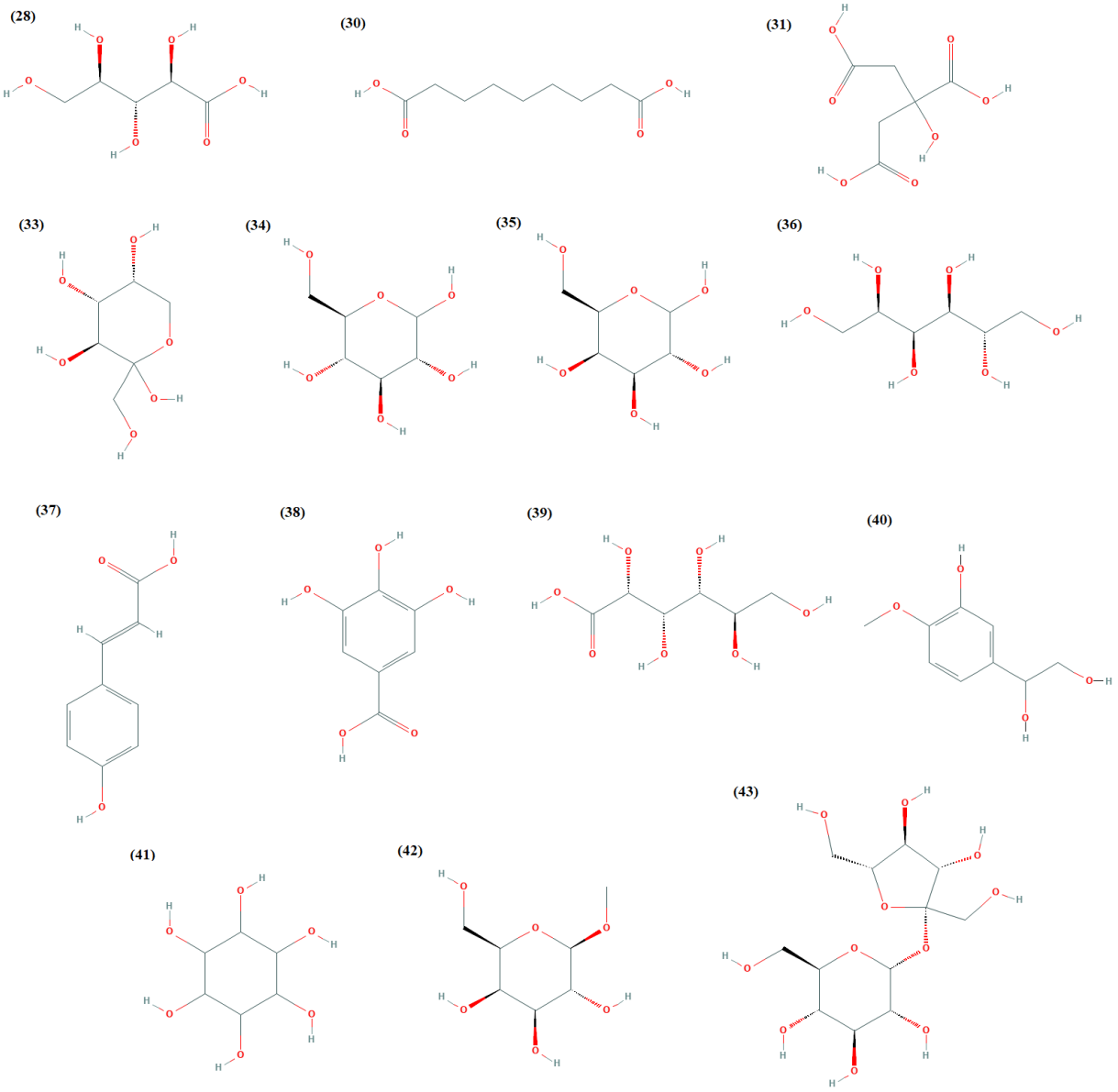


Figure 1.3 – Continuation.

Urea was detected at low levels in almost all species, except in MP, which did not present this compound. Besides, by this methodology, 3 phenolic compounds were also detected: Salicylic, Gallic, and *p*-Coumaric acids.

For hexane extracts, constituents were grouped into the following classes: alkanes, terpenoids, fatty acids, monoacylglycerols, vitamins, and saturated primary fatty alcohols. Terpenoids were subdivided into diterpenoid, triterpenoid, and phytosterol; and Fatty acids into polyunsaturated and saturated ones (Table 1.2; Figure 1.4).

Three alkanes were detected in hexane bamboo extracts: heptacosane, hentriacontane, and tetratetracontane. FC was the species that presented the highest levels of these substances (10%).

Regarding total terpenes, Phytol was the only diterpene observed, and the highest level was observed in OG (5%). However, four phytosterols have been detected, Campesterol, Stigmasterol, β-Sitosterol, and β-sitostenone. Another triterpene identified was the Friedelin, and was detected only in PM (3%). PM (19%), AA (19%), and MP (21%) showed similar levels of phytosterols.

Eleven fatty acids were detected including two polyunsaturated as linoleic and linolenic acids; as well as 8 saturated fatty acids were identified, such as Myristic, Palmitic, Margaric, Stearic, Arachidic, Behenic, Lignoceric, Hyenic, and Ceric acids. OG was the species that presented the highest levels of fatty acids among the studied species, with 80% of the hexane extract composition formed by fatty acids, being 50% of then polyunsaturated.

Two monoacylglycerols were detected, Monopalmitin and 2-Monostearin. OG was the species with the highest levels of these substances (5%).

Finally, three saturated primary fatty alcohols were detected: 1-Hexacosanol, Octacosyl, and 1-Triacontanol. The highest content of this group of substances was observed in FC (16%).

α-Tocopherol acetate, an important vitamin was detected in almost all studied species, except for OG.

Although it was possible to identify a diversity of compounds present in the hexane extracts of the studied species, a large part of the detected compounds was not identified, as we can see in table 1.2. More than half of the compounds detected in MP were not identified. Furthermore, it was clear that it would be necessary to use complementary identification methodologies for a robust analysis of nonpolar extracts.

Table 1.2 – Relative percentage of constituents detected by GC-EIMS in all bamboo nonpolar extracts. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.*

Peak	R.T. (min)	Constituent	Relative percentage (%)						Match NIST02	LRI	LRI GMD
			OG	PM	AA	FA	FC	MP			
44	12.70	NI-4	1.7	1.0	3.8	0.0	0.5	2.0	-	1848.27	*
45	12.96	Myristic acid (TMS)	0.0	0.0	1.1	0.7	0.0	0.0	846	1860.04	1845.7
46	13.61	NI-5	0.0	0.0	1.6	0.0	0.0	1.4	-	1889.42	*
47	16.80	NI-6	0.0	0.8	2.2	0.0	0.0	0.0	-	2038.39	*
48	16.98	Palmitic acid (TMS)	26.5	23.7	13.5	9.1	7.2	1.4	926	2049.06	2045.4
49	18.82	Margaric acid (TMS)	0.0	1.0	0.4	0.6	0.5	0.0	882	2152.58	2144.6
50	19.44	Phytol (TMS)	4.8	1.6	3.3	0.0	0.0	0.8	928	2186.26	2170.9
51	20.00	Linoleic acid (TMS)	19.7	4.7	6.6	3.0	1.8	0.0	936	2217.90	2209.9
52	20.11	Linolenic acid (TMS)	30.6	6.8	17.9	4.5	3.1	0.0	899	2224.10	*
53	20.58	Stearic acid (TMS)	3.9	7.0	2.6	3.6	3.1	0.0	893	2251.35	2243.5
54	23.69	NI-7	1.1	1.9	0.6	0.0	0.0	0.0	-	2437.44	*
55	23.86	Arachidic acid (TMS)	0.0	0.7	0.3	0.8	0.4	0.0	785	2448.40	2453.8
56	24.20	NI-8	0.9	1.4	0.4	0.0	0.0	0.0	-	2469.92	*
57	24.27	NI-9	0.6	1.6	0.5	0.0	0.0	0.0	-	2474.01	*
58	26.29	Monopalmitin (TMS)	2.3	0.0	0.8	0.2	0.4	0.0	803	2605.57	*
59	26.89	Behenic acid (TMS)	0.0	0.8	0.9	1.3	1.7	0.0	885	2644.73	2650.6
60	27.71	Heptacosane	0.0	1.8	0.9	2.1	2.4	0.7	840	2651.87	2699.2
61	28.33	NI-10	0.0	0.0	0.0	0.5	0.0	0.0	-	2673.34	*
62	28.74	2-Monostearin (2TMS)	2.3	0.0	0.3	0.0	0.0	0.0	776	2687.71	*
63	29.71	Lignoceric acid (TMS)	0.0	1.0	1.3	2.1	1.3	0.9	834	2822.87	2824.4
64	30.50	Hentriacontane	0.0	2.1	1.6	1.9	6.9	2.1	868	2851.91	*
65	31.05	Hyenic acid (TMS)	0.0	0.0	0.0	0.5	0.3	0.0	814	2872.56	2937.2
66	31.16	1-Hexacosanol	0.0	0.0	0.3	2.8	0.5	0.0	774	2876.49	*
67	31.46	NI-11	0.0	0.0	0.0	0.7	0.0	0.0	-	2887.82	*

Peak	R.T. (min)	Constituent	Relative percentage (%)						Match NIST02	LRI	LRI GMD
			OG	PM	AA	FA	FC	MP			
68	32.35	Ceric acid (TMS)	0.0	1.0	1.0	3.7	1.0	0.0	823	3022.08	3036.3
69	32.44	NI-12	0.0	0.0	0.0	1.2	0.4	0.0	-	3025.76	*
70	33.10	Tetratetracontane	0.0	1.2	3.0	0.5	1.0	1.7	707	3051.92	*
71	33.72	Octacosyl (TMS)	1.4	6.5	0.0	6.2	6.5	0.9	896	3076.48	*
72	34.00	NI-13	0.0	0.0	0.0	2.1	0.6	0.0	-	3087.58	*
73	34.30	α-Tocopherol acetate	0.0	0.6	0.5	1.3	0.2	1.8	795	3099.52	3200
74	34.50	NI-14	0.0	0.0	0.5	0.5	0.0	0.0	-	3203.85	*
75	34.84	NI-15	0.0	2.6	2.5	7.9	2.3	0.0	-	3211.22	*
76	34.90	NI-16	0.0	0.0	0.0	0.0	1.6	0.0	-	3212.34	*
77	35.15	Campesterol (TMS)	0.0	1.7	1.9	1.3	0.7	3.5	850	3217.80	3298.2
78	35.54	Stigmasterol (TMS)	0.5	6.5	3.3	2.9	2.1	5.6	893	3226.10	3319.3
79	35.67	NI-17	1.1	0.0	3.3	0.4	0.0	0.0	-	3228.67	*
80	35.78	NI-18	0.0	0.0	0.0	0.4	0.4	3.0	-	3231.00	*
81	35.87	NI-19	0.0	0.0	1.2	0.9	0.0	1.6	-	3233.00	*
82	36.08	1-Triacontanol (TMS)	0.0	0.9	1.8	2.2	9.0	0.0	799	3237.53	*
83	36.24	β-Sitosterol (TMS)	1.5	10.7	13.8	7.3	6.2	12.1	872	3240.92	3385.8
84	36.33	NI-20	1.1	0.6	0.0	0.7	3.8	2.1	-	3242.73	*
85	36.40	NI-21	0.0	1.7	2.6	2.6	0.0	0.0	-	3244.34	*
86	36.56	NI-22	0.0	1.3	0.7	0.0	0.0	3.5	-	3247.70	*
87	36.73	NI-23	0.0	0.4	0.9	1.7	1.4	0.9	-	3251.37	*
88	36.87	NI-24	0.0	0.7	0.0	1.4	0.5	2.9	-	3254.33	*
89	37.04	NI-25	0.0	0.0	0.0	0.7	0.0	0.0	-	3257.93	*
90	37.17	NI-26	0.0	1.1	1.0	2.0	2.6	0.0	-	3260.71	*
91	37.28	NI-27	0.0	0.0	0.0	0.6	2.7	0.0	-	3263.04	*
92	37.42	β-sitostenone	0.0	0.0	0.0	4.7	0.0	0.0	815	3265.83	*
93	37.53	NI-28	0.0	0.0	0.0	6.3	22.4	2.6	-	3268.29	*
94	37.79	NI-29	0.0	0.0	1.3	1.9	0.9	0.0	-	3273.88	*
95	37.99	NI-30	0.0	1.4	0.0	0.7	1.1	13.9	-	3278.14	*

Peak	R.T. (min)	Constituent	Relative percentage (%)						Match NIST02	LRI	LRI GMD
			OG	PM	AA	FA	FC	MP			
96	38.27	Friedelin	0.0	3.1	0.0	0.0	0.0	0.0	830	3283.90	*
97	38.34	NI-31	0.0	0.0	0.0	0.5	0.6	32.9	-	3285.41	*
98	38.66	NI-32	0.0	0.0	0.0	0.6	0.3	0.0	-	3292.32	*
99	38.84	NI-33	0.0	0.0	0.0	0.9	1.2	0.0	-	3296.04	*
100	39.21	NI-34	0.0	0.0	0.0	0.6	0.0	0.0	-	3608.43	*
101	39.53	NI-35	0.0	0.0	0.0	0.3	0.1	1.7	-	3623.72	*
102	39.59	NI-36	0.0	0.0	0.0	0.5	0.0	0.0	-	3626.33	*
		Alkane	**0.0**	**5.1**	**5.5**	**4.5**	**10.3**	**4.6**			
		Terpenoid (total):	**6.9**	**23.7**	**22.3**	**16.2**	**9.0**	**21.9**			
		Diterpenoid	**4.8**	**1.6**	**3.3**	**0.0**	**0.0**	**0.8**			
		Triterpenoid	**0.0**	**3.1**	**0.0**	**0.0**	**0.0**	**0.0**			
		Phytosterol	**2.1**	**19.0**	**19.0**	**16.2**	**9.0**	**21.1**			
		Fatty acid (total):	**80.6**	**46.7**	**45.6**	**29.9**	**20.4**	**2.3**			
		Polyunsaturated	**50.3**	**11.5**	**24.5**	**7.6**	**4.9**	**0.0**			
		Saturated	**30.3**	**35.2**	**21.1**	**22.3**	**15.5**	**2.3**			
		Monoacylglycerol	**4.6**	**0.0**	**1.1**	**0.2**	**0.4**	**0.0**			
		Saturated primary fatty alcohol	**1.4**	**7.4**	**2.1**	**11.2**	**16.1**	**0.9**			
		Vitamin	**0.0**	**0.6**	**0.5**	**1.3**	**0.2**	**1.8**			
		Unkown	**6.5**	**16.5**	**23.0**	**36.7**	**43.6**	**68.4**			

-: data with any hit in the used library ***:** values not available

LRI: Linear Retention Index. **LRI GMD**: Linear Retention Index available in Golm Metabolome Database.

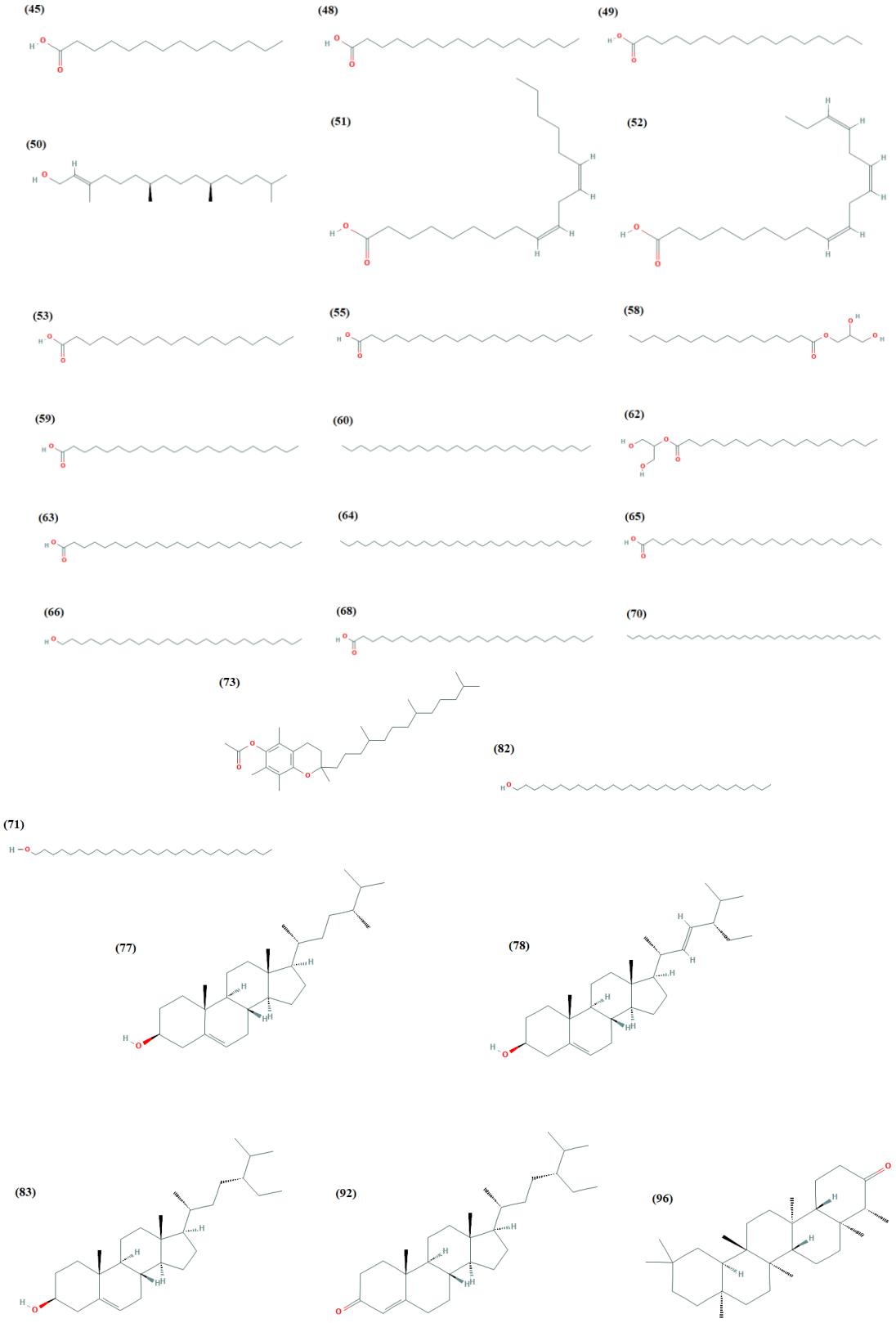


Figure 1.4 – Compounds identified in the nonpolar extracts. The numbers above each chemical structure correspond to those shown in Table 1.2. Chemicals structures source: Pubchem, 2020.

Polar extracts of the Brazilian bamboo species showed a great diversity of constituents, as amino acids, carbohydrates, and phenolic acids, with important nutritional value.

Free amino acids are building blocks in protein synthesis and they also have critical roles in plant growth and physiology. They perform essential functions in both primary and secondary plant metabolism (Buchanan et al. 2015).

Some amino acids are important in nitrogen (N) uptake and transport from sources to sink parts. They are also important as precursors to phytohormones and to several secondary compounds involved in the plant interactions with their abiotic and biotic environments (Buchanan et al. 2015). Furthermore, amino acids are also extremely important for humans, because they have the same role in protein synthesis; they also constitute the building blocks for several other biosynthetic pathways, being important as key precursors to animal hormones synthesis and others substances of physiological importance, like glutathione and serotonin (Dato et al. 2019).

However, many of these amino acids are not synthesized by animals, being called essential amino acids for human diet, since they must be ingested through the diet; are they: phenylalanine, tyrosine, tryptophan, valine, threonine, methionine, leucine, isoleucine, lysine, and histidine. They are responsible for the regulation of glucose and lipid metabolism and energy balance, increasing mitochondrial biogenesis and maintaining the immune dynamic-homeostasis (Dato et al. 2019).

Due to their great importance in the human and animal diet, amino acids are also widely used as a nutritional supplement in foods, beverages, pharmaceuticals, and animal feeds.

In general, most studies on the nutritional value of bamboo refer to young shoots, because they can be consumed fresh, fermented, or canned. They are rich in nutrient as proteins, carbohydrates, minerals, fiber, but have low contents of fat and sugars (Chongtham et al. 2011).

Besides the shoots, bamboo culms present nutritional value and can be consumed as flour. Felisberto et al. (2017) evaluated the physicochemical and technological characteristics for food application of the flour produced from culms of *Dendrocalamus asper*. The authors observed a great potential of employing this flour as a new ingredient for food products, improving the intake of insoluble fiber.

Bamboo leaves extracts are used as an antioxidant additive in various industrialized foods in China (Ministry of Health 2003). Moreover, some fried foods that were pre-treated with these bamboo extracts showed significantly reduction in the formation of acrylamide, which is a toxic and carcinogenic compound formed during food frying process (Zhang et al. 2007).

The hexane leaf extracts of the Brazilian bamboos also showed a great diversity of substances, such as phytosterols and polyunsaturated fatty acids which were the major bioactive components in these species.

The most commonly phytosterols in plants are β-sitosterol, campesterol, and stigmasterol (López-García et al. 2018). Phytosterols occur in all plant tissues, and can be found in five common forms: free sterols, fatty-acid esters (steryl esters), steryl glycosides, acylated steryl glycosides, and hydroxycinnamic acid steryl esters. More than 200 different types of phytosterols have been reported in plant species (López-García et al. 2018).

Miras-Moreno et al. (2016) described several important pharmacological properties attributed to phytosterols, among them anti-inflammatory, antidiabetic, and antitumoral effects. According to them, phytosterols have had special interest because of their cholesterol-lowering activities and their protective effect against cardiovascular diseases.

As seen in table 1.2 another group of compounds very expressive in hexane extracts were fatty acids. In general, the studied species showed high levels of saturated fatty acids, with the exception of OG which presented high levels of polyunsaturated fatty acids.

Lipids serve many functions in plants; they represent a substantial chemical reserve of free energy. Because fatty acids are substantially more reduced organic molecules than carbohydrates, fatty acid oxidation has a higher potential for producing energy (Buchanan et al. 2015).

A major fraction of the fatty acids in plants are the polyunsaturated ones as linoleic and α-linolenic acids (Buchanan et al. 2015). In mammalian tissues these polyunsaturated fatty acids cannot be synthesized endogenously, and they must be obtained from the diet (Sardesai, 1992).

Therefore, the bamboo species here studied could be an important source of polyunsaturated fatty acids, especially OG.

By GC-EIMS analysis it was possible to identify a great diversity of metabolites. Some of these metabolites stood out in relation to their contents, as soluble sugars, fatty acids, phytosterols and organic acids (Figure 1.5).

For almost all species the most abundant constituents were soluble sugars, as Fructose and Glucose, only OG showed high levels of Sucrose. It is possible to observe that the unidentified compounds of the hexane extracts represent a large percentage of the chemical composition of these extracts, especially for FA, FC, and MP, being important to use another technique of compound identification for nonpolar extracts (Figure 1.5).

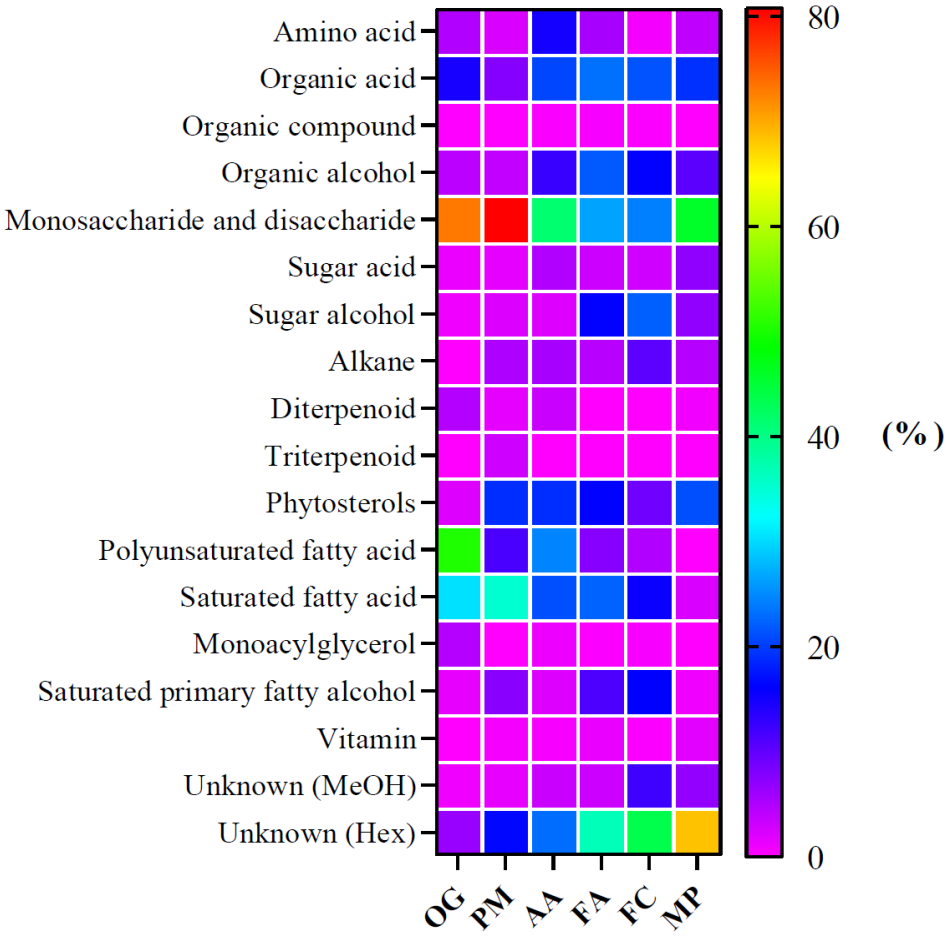


Figure 1.5 – Heat map of the relative percentage of the metabolite classes analyzed by GC-EIMS in six Brazilian bamboo species. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.*

1.3.4. HPLC-ESIMS/MS analysis

By HPLC-ESIMS/MS analysis it was identified 115 constituents from hydroethanol and aqueous extracts, using the GNPS online platform as reference (Table 1.3).

GNPS provides the ability to analyze a data set and to compare it to all available data in this platform, which combines a catalog of over 221,000 MS/MS reference library spectra from 18,163 compounds. These include libraries, such as MassBank, ReSpect, and NIST, as well as spectral libraries created for GNPS (GNPS-Collections) and spectra from the NP community (GNPS-Community) (Wang et al. 2016).

These consensus MS/MS spectra are then simplified as vectors in a multidimensional normalized space where each dimension corresponds to an *m*/z value and its respective intensity.

These vectors are then used to calculate a cosine score between every possible pair of consensus MS/MS spectrum, which allows the determination of the degree of spectral similarity between them (ranging from 0 to 1.1). Cosine score is the expression of the angles between a pair of vectored MS/MS spectra, therefore, the closer to 1, the greater the index of similarity between the experimental spectrum and the library (Quinn et al. 2017).

A variety of compounds belonging to different classes of metabolites have been detected. Among the phenolic compounds, flavonoids, chalcones, chromenes, anthraquinones, aurones, lignans, anthocyanins, and hydroxycinnamic acid derivatives were identified (Table 1.3).

Different terpenoid substances have also been observed, such as diterpenes, cardenolides, phytosterols, sesquiterpene lactones, and saponins. Some nitrogen compounds were also detected, as free and conjugated amino acids, alkaloids, as well as purine nucleoside and nucleotides (Table 1.3).

Other groups of substances have also been detected, such as glycerophospholipids, glycerophosphoinositols, glycerides, sphingolipids, monosaccharides, disaccharides, and vitamins (Table 1.3).

Although a wide variety of compounds from different classes were observed, flavonoids were the most expressive, being detected 35 flavones, 11 flavonols, and 2 isoflavones.

Some of these flavones were observed in all studied species, such as Vicenin, Schaftoside, Isovitexin**,** and 2',5,6-trimethoxyflavone. However, some were more restricted, such as Swertisin, Diosmin, Neodiosmin, Apigetrin, Apigenin 4'-*O*-glucoside, Tricin 7-glucoside, and Chrysoeriol 7-*O*-Neohesperidoside, which were detected only in PM. As well as the flavones 2"-*O*-beta-*D*-Xylopyranosylorientin, *C*-Hexosyl-chrysoeriol *O*-pentoside**,** the isoflavone Afromosin 7-*O*-glucoside, the flavonol Quercetin 3,4'-*O*-diglucoside, and the anthocyanidin Peonidin 3-galactoside were detected only in AA. The flavone Methoxy-myricetin-*O*-hexosyl-*O*-hexoside and the flavonol Hyperoside were observed only in FA; while the flavonol Pectolinarin were detected in FC. Finally, the flavones Rhoifolin and Vitexin 4"-*O*-glucoside were observed only in MP.

As described previously, flavones, especially *C*-glycosylated, have already been reported for other bamboo species. Some of them, as Vitexin, Isovitexin, and Isoorientin, were detected in *Phyllostachys nigra, Pleioblastus argenteostriatu, Sasa kurilensis,* and *Bambusa nutans* (Van Hoyweghen et al. 2014; Ni et al. 2012; Yang et al. 2017; Pande et al. 2018).

Even though it is more unusual, Quercetin was previously reported for *Aulonemia aristulata* (Grombone-Guaratini et al. 2009), and in the present study it was detected Quercetin 3,4'-*O*-diglucoside, corroborating the presence of flavonols in this species.

Regarding other classes of metabolites, only seven substances were detected in all studied species, the vitamin Riboflavin, the aliphatic alcohol Docosanol, the chromen (8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl) (*Z*)-2-methylbut-2-enoate, the dihydrochalcone Avobenzone, the sphingolipid N-(11*Z*-eicosenoyl)-1-β-galactosyl-4E,14*Z*-sphingadienine**,** the cardenolide Sarmentoside B, and lastly the pigment Pheophytin**.**

However, some of these compounds have also been detected in a single species, such as the diterpen Sclareol, the glyceride 2-Linoleoylglycerol, the glycerophosphoinositol 1-Hexadecanoyl-sn-glycero-3-phospho-(1'-*myo*-inositol), and the lignan Liriodendrin, detected only in OG.

The lignan Liriodendrin was also reported in *Bambusa emeiensis* (Luo et al. 2015), a tropical woody bamboo. In the present work this compound was detected only in *Olyra glaberrima,* an herbaceous species.

Some of the hydroxycinnamic acid derivatives as Isochlorogenic acid b, 4-*p*-Coumaroylquinic acid, 6-*O*-Caffeoylarbutin**,** and phenylglycoside Juniperoside III, the phenylamide Spermidine-diferuloyl, the chromen Apaensin, the glycerolipid 1-hexadecanoyl-2-(9Z,12Z,15Z-octadecatrienoyl)-sn-glycerol, and the sucrose ester Sibiricose A1 were detected in PM.

The amino acid L-Tryptophan and the glycerophospholipid 1-palmitoyl-2-homo-g-linolenoyl-sn-glycero-3-phosphocholine were detected in AA. The acetogenin Avocadene 4-acetate, the glycerophospholipids 2-Oleoyl-sn-glycero-3-phosphocholine and 1-Stearoyl-2-linoleoylphosphatidylcholine, the lignan Eudesmin, and the sesquiterpene lactone Costunolide, were observed in FA.

Table 1.3 – Constituents from hydroethanol **(E)** and aqueous **(A)** extracts analyzed by HPLC-ESIMS/MS in six Brazilian bamboo species. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata*; **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora*.

Peak	R.T. (min)	Constituent	Relative percentage (%)												Cosine
			OGE	OGA	PME	PMA	AAE	AAA	FAE	FAA	FCE	FCA	MPE	MPA	
103	4.42	Deoxyfructosazine	0.0	0.0	1.7	0.0	0.1	0.0	0.9	0.0	0.0	0.0	0.6	0.0	0.77
104	6.56	Hyperoside	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.80
105	7.82	Cordycepin	0.0	1.0	0.0	0.0	0.0	1.0	0.6	0.0	0.0	0.0	0.0	0.0	0.95
106	8.02	Inosine	0.5	0.0	0.0	0.4	0.0	0.0	0.0	0.9	1.1	0.0	0.0	0.0	0.93
107	14.52	Succinoadenosine	1.5	1.8	0.0	0.0	0.2	0.2	0.7	0.7	2.1	0.8	0.0	0.3	0.88
108	15.73	Isochlorogenic acid b	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.71
109	15.95	Tryptophan N-glucoside	1.1	13.4	0.2	6.2	0.4	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.82
110	16.25	(3R,5S)-4-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,3,5-trihydroxycyclohexane-1-carboxylic acid	0.0	0.0	0.1	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.73
111	16.35	Chlorogenic acid	0.0	0.0	0.2	0.7	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.77
112	16.44	4-*p*-Coumaroylquinic acid	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.81
113	16.87	L-Tryptophan	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.77
114	18.26	Kaempferol 3-glucuronide	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.8	0.85
115	18.42	Luteolin 7-glucuronide	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.85
116	19.62	Licoagroside B	0.0	0.0	0.0	0.0	0.1	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.89
117	20.94	Riboflavin	0.4	1.8	0.0	0.3	0.0	4.3	1.2	0.6	4.1	0.9	4.1	1.8	0.86
118	22.14	Apigenin 6,8-digalactoside	0.4	1.4	0.2	0.4	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.77
119	23.71	Arillatose B	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.85
120	23.96	Vicenin	1.0	8.8	8.8	17.4	14.3	24.6	0.6	0.8	0.0	8.1	29.1	45.4	0.94
121	25.70	Sibiricose A1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.75
122	25.71	Corymboside	0.0	1.3	0.0	0.0	0.0	1.9	0.7	0.5	0.3	0.0	0.5	0.1	0.93

Peak	R.T. (min)	Constituent	Relative percentage (%)												Cosine
			OGE	OGA	PME	PMA	AAE	AAA	FAE	FAA	FCE	FCA	MPE	MPA	
123	26.47	(E)-N-(4-acetamidobutyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide	1.0	0.0	0.0	0.0	0.8	7.1	0.0	0.0	0.0	0.0	1.8	1.1	0.73
124	27.39	5,7-dihydroxy-2-phenyl-6,8-bis[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one	0.0	0.0	9.1	9.1	0.0	0.0	25.9	27.8	0.0	18.7	20.6	15.0	0.72
125	28.11	Saponarin	5.0	8.8	0.7	3.4	0.1	0.0	1.7	3.9	0.0	0.0	0.0	0.0	0.86
126	29.14	Schaftoside	0.2	2.6	0.7	0.2	0.1	1.2	6.3	4.8	1.9	1.2	3.0	8.5	0.93
127	29.75	Vitexin -4"-*O*-glucoside	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.80
128	29.77	8-[4,5-dihydroxy-6-(hydroxymethyl)-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]-5.7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one	0.5	1.7	1.9	5.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.97
129	30.24	2"-*O*-beta-D-Xylopyranosylorientin	0.0	0.0	0.0	0.0	0.2	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.88
130	30.91	Swertisin	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.73
131	30.92	Isoorientin	0.0	0.0	0.8	2.7	0.1	0.1	4.3	5.9	0.8	6.3	1.1	0.9	0.91
132	31.42	Tricin 5-glucoside	0.0	0.0	12.4	13.5	3.9	2.6	23.6	22.4	12.2	8.6	2.2	3.7	0.96
133	32.26	Violanthin	0.0	0.0	0.0	0.0	0.2	0.0	0.5	0.0	0.4	0.0	0.0	0.0	0.79
134	32.41	Isovitexin	2.2	2.6	0.2	0.1	0.2	0.9	0.7	0.0	7.6	10.3	1.6	3.1	0.89
135	32.69	Quercetin 3,4'-*O*-diglucoside	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.89
136	33.03	Isovitexin 2"-*O*-arabinoside	0.0	0.0	0.0	0.0	16.2	15.8	0.0	0.0	0.0	0.0	0.7	1.1	0.93
137	33.61	Syringetin-3-*O*-glucoside	0.7	2.7	4.2	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.6	1.2	0.79
138	33.73	*C*-Hexosyl-chrysoeriol *O*-hexoside	0.2	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.0	0.83
139	33.87	6-hydroxy-3-[3-hydroxy-4-[3,4.5-trihydroxy-6-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyphenyl]-5,7-dimethoxychromen-4-one	0.0	0.0	0.0	0.0	2.8	4.4	0.0	0.0	10.1	19.8	0.0	0.0	0.93
140	33.87	Vitexin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.9	11.1	0.0	4.3	0.3	0.82
141	33.96	Afzelin	0.0	0.0	0.1	0.3	0.3	3.1	0.2	0.0	0.0	0.0	0.0	0.0	0.82
142	34.02	6-*O*-Caffeoylarbutin	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.71

Peak	R.T. (min)	Constituent	Relative percentage (%)												Cosine
			OGE	OGA	PME	PMA	AAE	AAA	FAE	FAA	FCE	FCA	MPE	MPA	
143	34.48	5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-bis(3,4,5-trihydroxyoxan-2-yl)chromen-4-one	1.6	6.8	19.0	23.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.80
144	34.88	*C*-Hexosyl-chrysoeriol *O*-pentoside	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.85
145	36.07	(2Z)-4,6-dihydroxy-2-[(4-hydroxy-3,5-dimethoxyphenyl)methylidene]-1-benzofuran-3-one	0.0	0.0	7.7	0.0	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.1	0.72
146	37.73	Isoscoparin	0.9	0.0	0.0	0.0	0.0	0.0	0.9	0.7	0.0	0.0	0.0	0.0	0.79
147	39.49	Rhamnetin 3-sophoroside	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.4	0.0	0.0	0.0	0.87
148	40.39	Peonidin-3-glucoside	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.72
149	40.45	Chrysoeriol	1.4	0.0	1.7	0.0	0.1	0.0	1.1	0.0	3.8	0.0	0.0	0.0	0.78
150	40.52	*C*-Hexosyl-chrysoeriol	0.0	0.0	6.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.73
151	40.52	Peonidin 3-galactoside	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.90
152	40.61	Liriodendrin	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.79
153	44.46	Feruloyltyramine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.84
154	44.68	5-hydroxy-7-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2-[4-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyphenyl]chromen-4-one	0.0	0.0	1.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.93
155	45.54	Rhoifolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.86
156	45.55	3-(alpha-L-Rhamnopyranosyloxy)-8-(beta-D-glucopyranosyloxy)-3',4',5-trihydroxy-7-methoxyflavone	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	3.4	3.6	0.0	0.0	0.78
157	45.65	Diosmin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.72
158	45.66	Neodiosmin	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.98
159	45.73	Chrysoeriol 7-*O*-Neohesperidoside	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.74
160	45.73	Apigetrin	0.0	0.0	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.90
161	45.92	Apigenin 4'-*O*-glucoside	0.0	0.0	1.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.97
162	46.18	Matairesinol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.0	10.0	0.0	0.0	0.92
163	46.24	Pectolinarin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.77

Peak	R.T. (min)	Constituent	Relative percentage (%)												Cosine
			OGE	OGA	PME	PMA	AAE	AAA	FAE	FAA	FCE	FCA	MPE	MPA	
164	46.32	6-hydroxy-3-[3-hydroxy-4-[3.4.5-trihydroxy-6-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyphenyl]-5,7-dimethoxychromen-4-one	0.0	0.2	0.0	0.0	0.2	0.0	0.3	0.0	3.7	0.0	0.0	0.0	0.90
165	46.40	3-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxychromen-4-one	0.0	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3	0.0	0.0	0.90
166	47.19	Afromosin 7-*O*-glucoside	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.90
167	47.21	Tricin 7-glucoside	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.77
168	47.37	Spermidine-diferuloyl	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.84
169	47.63	Tricin 7-glucuronide	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.3	0.4	0.4	0.0	0.0	0.83
170	54.23	Spinacetin 3-gentiobioside	0.0	5.6	0.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	5.5	2.6	0.87
171	54.63	4-*O*-Acetyl-beta-D-fructofuranosyl 6-*O*-[(2E)-3-phenyl-2-propenoyl]-alpha-D-glucopyranoside	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.74
172	56.13	Costunolide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.70
173	60.04	3-*p*-coumaroylquinic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.74
174	63.08	Apaensin	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.70
175	65.62	Adenosine 5'-diphosphate	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.72
176	67.45	Monolinolenin	1.1	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.72
177	68.31	Methoxy-myricetin-*O*-hexosyl-*O*-hexoside	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.73
178	69.87	2-Linoleoylglycerol	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.74
179	69.92	Glc-Glc-octadecatrienoyl-sn-glycerol	1.0	0.0	0.1	0.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.84
180	70.59	1-Hexadecanoyl-sn-glycero-3-phospho-(1'-myo-inositol)	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.73
181	76.30	2',5,6-trimethoxyflavone	1.6	2.6	0.0	0.1	0.5	0.2	0.4	0.3	0.3	0.2	0.0	1.2	0.89
182	78.59	Docosanol	5.6	0.5	1.0	0.5	0.0	0.4	0.0	0.8	0.4	0.0	0.2	0.1	0.97
183	78.65	1-(2E,4E-octadecadienoyl)-sn-glycero-3-	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.7	0.0	0.0	0.0	0.87

Peak	R.T. (min)	Constituent	Relative percentage (%)												Cosine
			OGE	OGA	PME	PMA	AAE	AAA	FAE	FAA	FCE	FCA	MPE	MPA	
		phosphocholine													
184	80.09	1-hexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)	0.7	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.87
185	80.10	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.85
186	80.12	(8,8-dimethyl-2,10-dioxo-9H-pyrano[2.3-f]chromen-9-yl) (Z)-2-methylbut-2-enoate	0.4	3.0	0.3	0.7	0.1	0.3	1.4	0.1	0.3	0.0	0.2	0.3	0.94
187	80.33	2-Palmitoyl-sn-glycero-3-phosphocholine	11.5	0.0	0.4	0.0	0.2	0.0	0.0	0.0	0.9	0.0	0.7	0.0	0.91
188	80.89	1-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)	0.9	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.87
189	81.13	2-Oleoyl-sn-glycero-3-phosphocholine	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.87
190	81.20	2-octadecanoyl-sn-glycero-3-phosphocholine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.78
191	81.66	Juniperoside III	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.71
192	81.70	Polanrazine B	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.7	0.0	1.3	0.0	0.79
193	82.18	1,2-dia-linolenoyl-rac-glycerol	16.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.75
194	83.53	Enniatin B	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.83
195	83.95	1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)	0.0	0.0	0.0	0.3	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.77
196	84.00	1-Stearoyl-2-linoleoylphosphatidylcholine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.74
197	84.44	Campesterol	0.0	0.0	0.1	0.0	0.0	0.0	1.2	0.0	0.8	1.0	0.0	0.0	0.80
198	85.03	*β*-Sitosterol	0.0	0.0	0.4	0.0	0.0	0.1	0.0	0.6	0.0	0.0	1.8	0.9	0.75
199	85.99	Avobenzone	0.0	0.9	0.0	0.5	0.0	3.8	2.5	0.0	0.0	0.8	0.8	0.9	0.94
200	86.26	1-hexadecanoyl-2-(9Z,12Z,15Z-octadecatrienoyl)-sn-glycerol	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.71
201	86.67	Galactosylceramide(d18:2/18:1)	0.0	0.0	0.3	0.0	7.5	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.88
202	87.25	1-palmitoyl-2-homo-g-linolenoyl-sn-glycero-3-phosphocholine	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.93
203	87.65	N-(11Z-eicosenoyl)-1-β-galactosyl-4E,14Z-sphingadienine	0.0	1.2	2.7	1.6	6.3	1.9	0.9	0.0	3.7	1.8	1.6	1.6	0.88

Peak	R.T. (min)	Constituent	Relative percentage (%)												Cosine
			OGE	OGA	PME	PMA	AAE	AAA	FAE	FAA	FCE	FCA	MPE	MPA	
204	87.67	N-(11Z-eicosenoyl)-4E,14Z-sphingadienine	0.0	0.0	1.4	0.0	7.7	0.0	1.8	0.0	1.2	0.0	0.9	0.0	0.86
205	88.07	Eudesmin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.85
206	88.65	Galactosylceramide (d18:2/22:1)	0.0	0.0	0.7	0.0	18.2	0.0	0.0	0.0	2.5	0.0	1.1	0.0	0.82
207	90.31	Chiococcasaponin IV	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.72
208	90.73	N-(octadecanoyl)-sphinganine	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.75
209	91.05	Koaburside	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	1.8	0.71
210	91.07	Avocadene 4-acetate	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.72
211	91.13	1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.75
212	91.22	Isosulochrin	0.0	0.0	0.4	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.79
213	91.25	α-Tochopheryl acetate	0.0	0.0	0.0	0.0	0.0	0.0	0.8	2.0	2.1	0.0	2.8	0.0	0.88
214	91.27	Sarmentoside B	8.8	14.0	1.5	0.2	8.2	10.5	5.3	5.2	2.7	2.1	1.4	1.6	0.76
215	92.06	Bidwillon A	0.0	10.9	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.71
216	93.31	Pheophytin	15.3	0.0	4.6	0.0	2.3	0.0	5.7	0.0	2.8	0.0	3.0	0.0	0.92
217	93.83	Sclareol	14.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.74

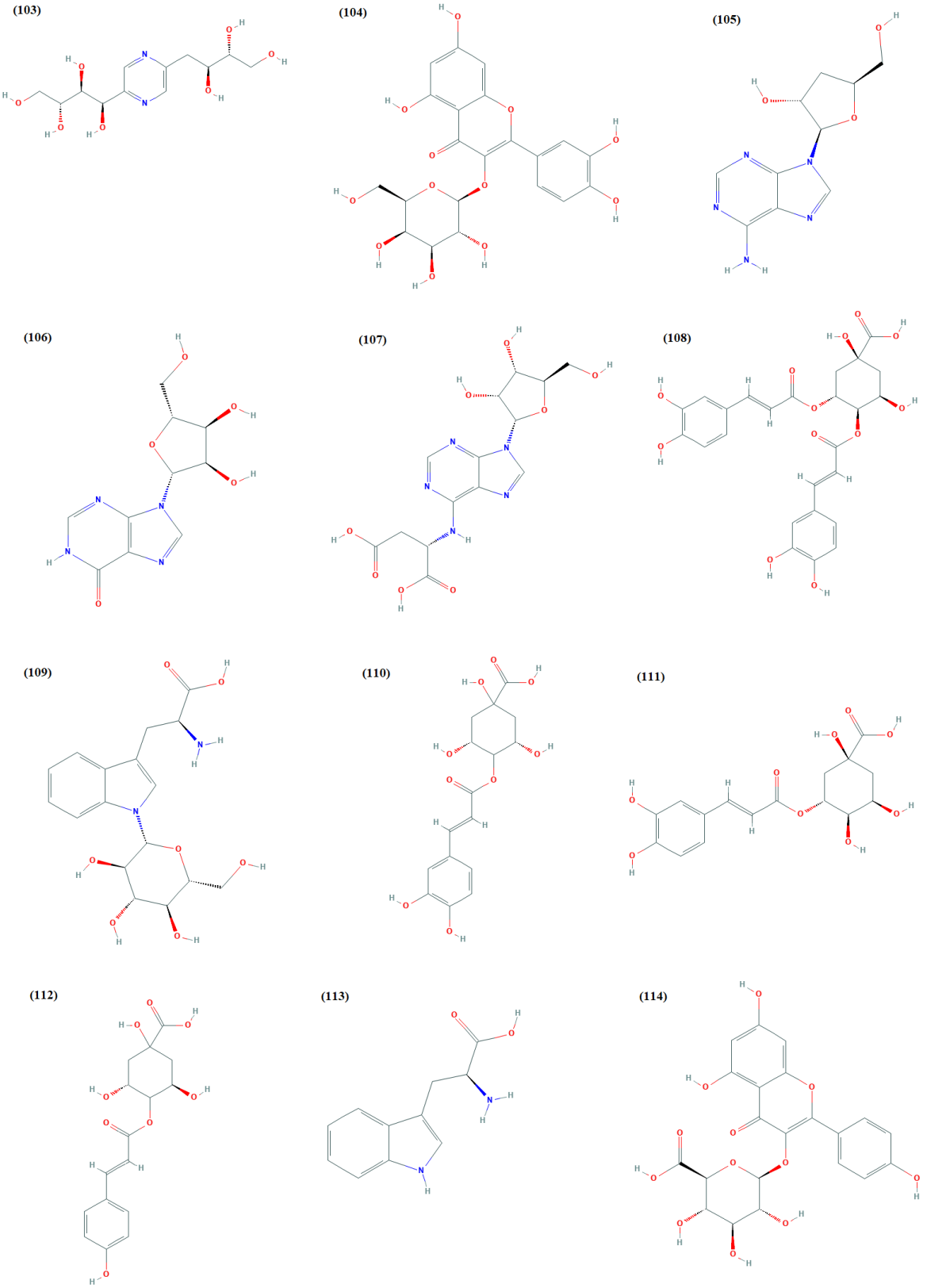
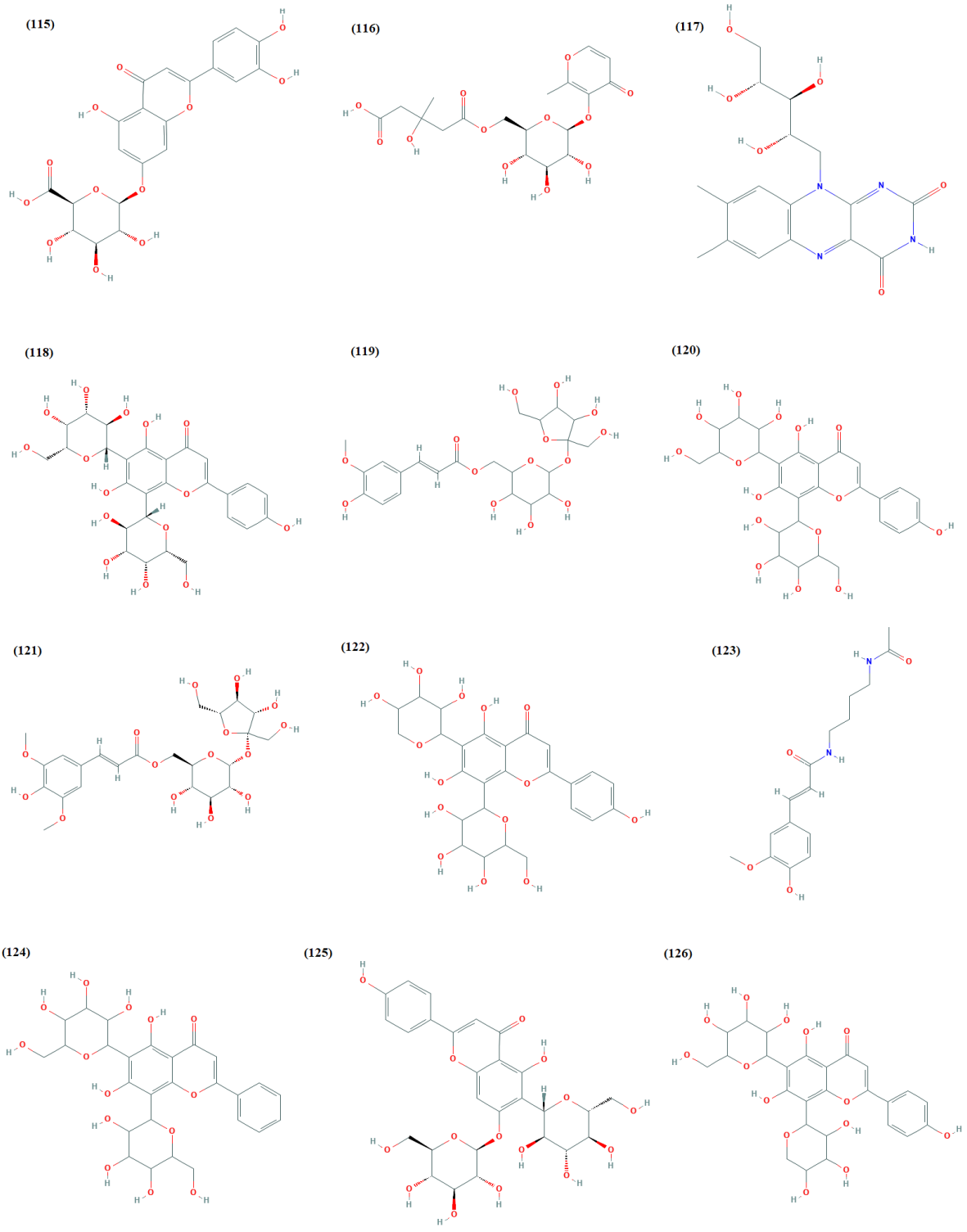


Figure 1.6 – Compounds identified in the hydroethanol and aqueous extracts of six Brazilian bamboo species. The numbers above each chemical structure correspond to those shown in Table 1.3. Chemicals structures source: Pubchem, 2020.



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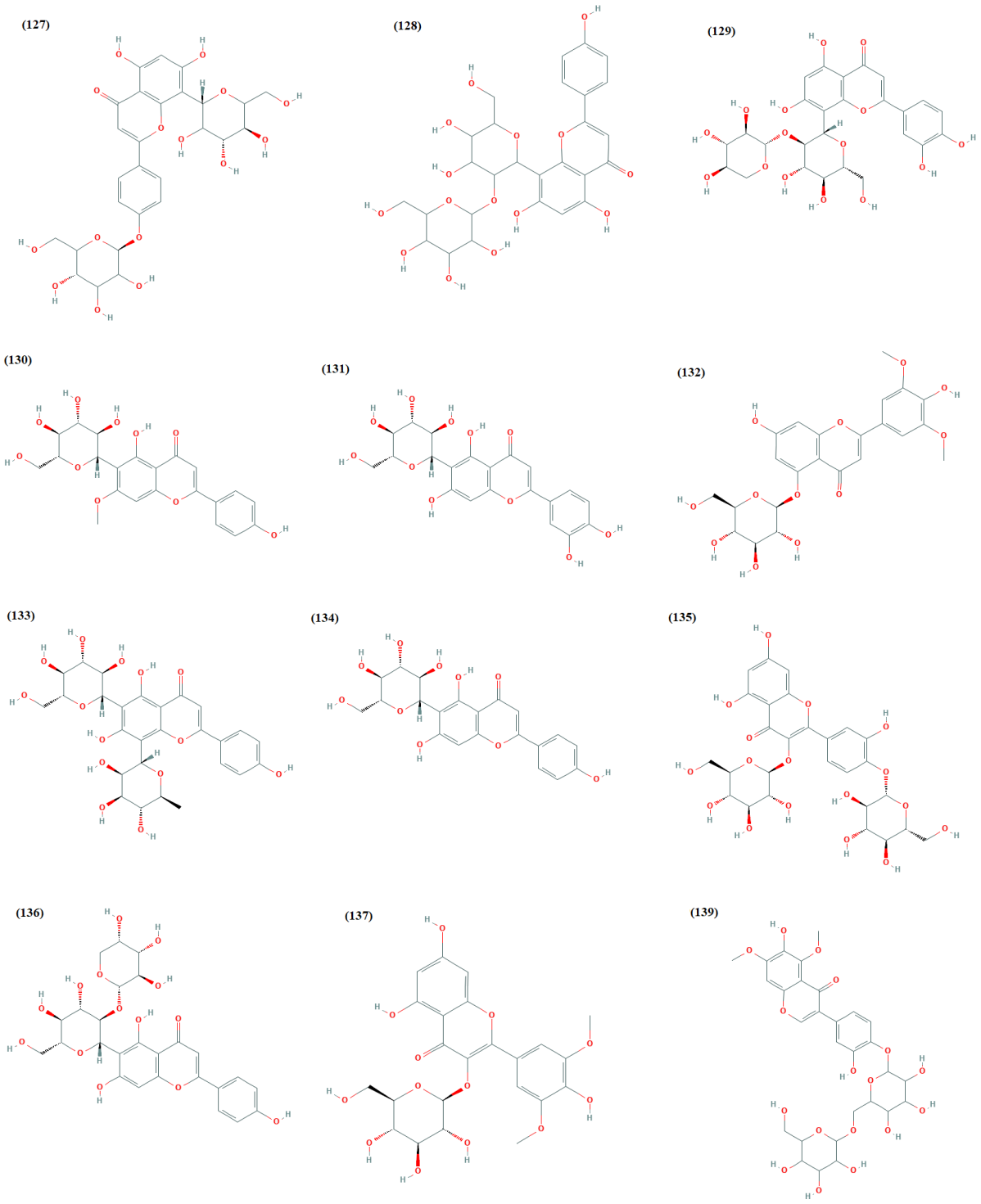

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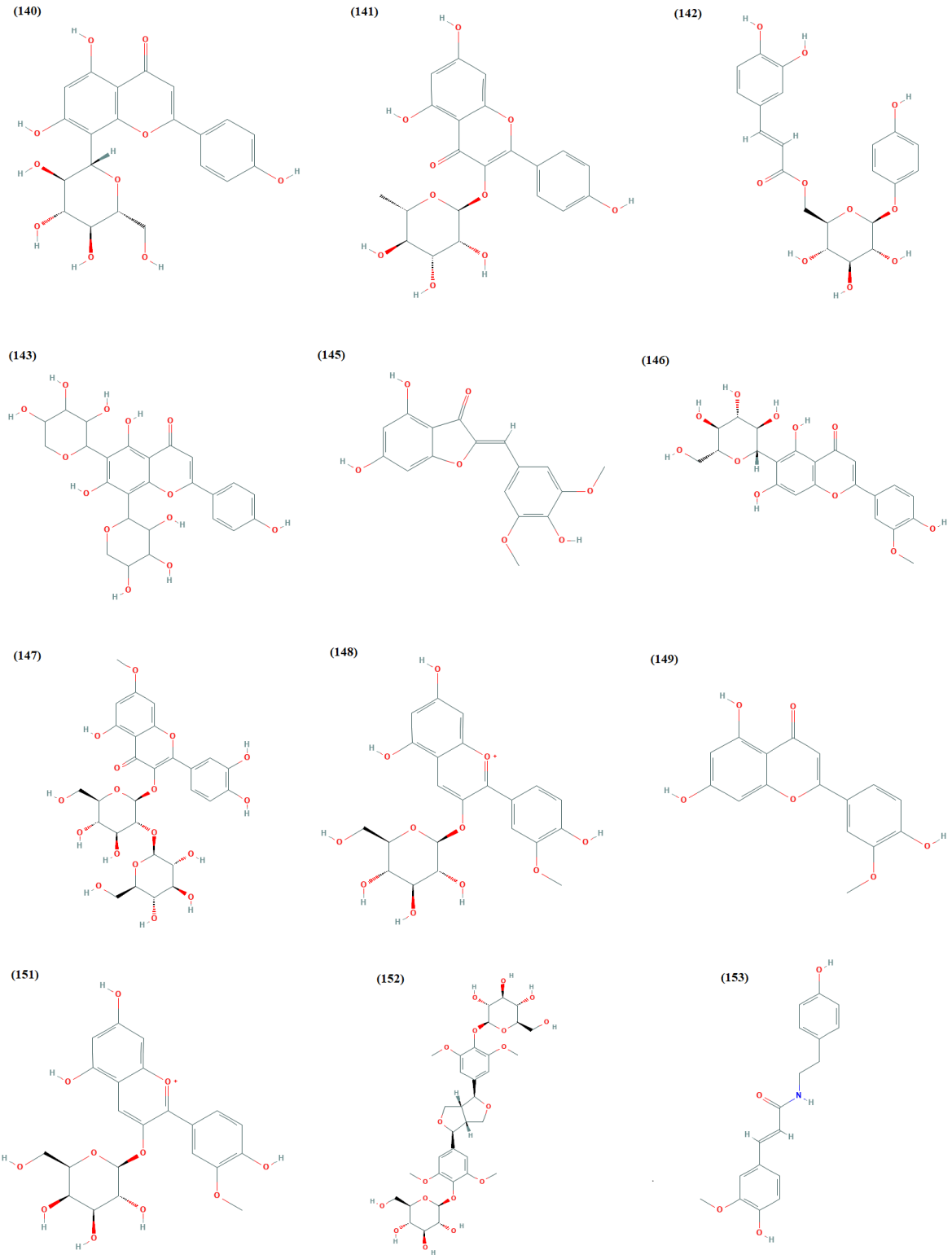

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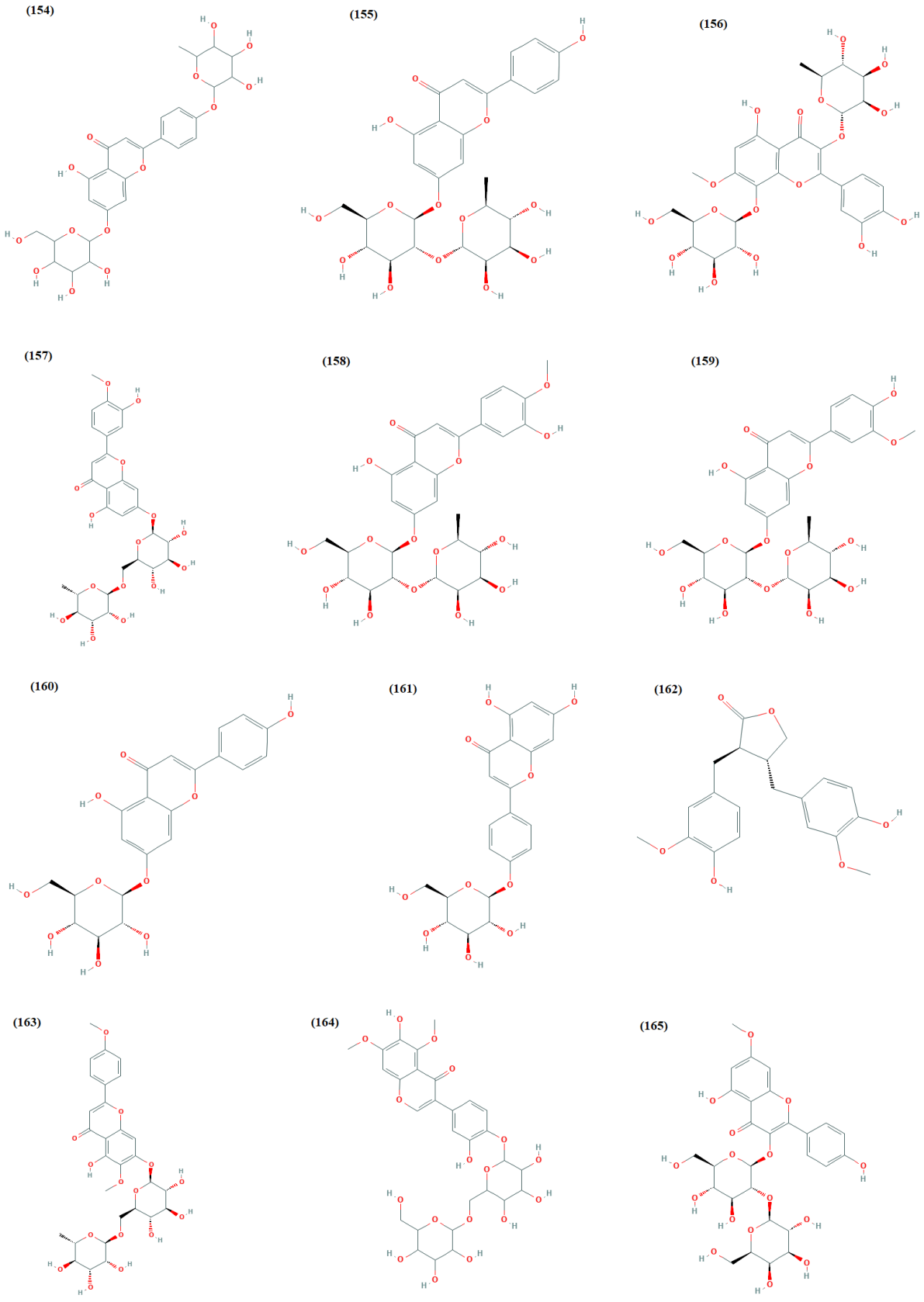

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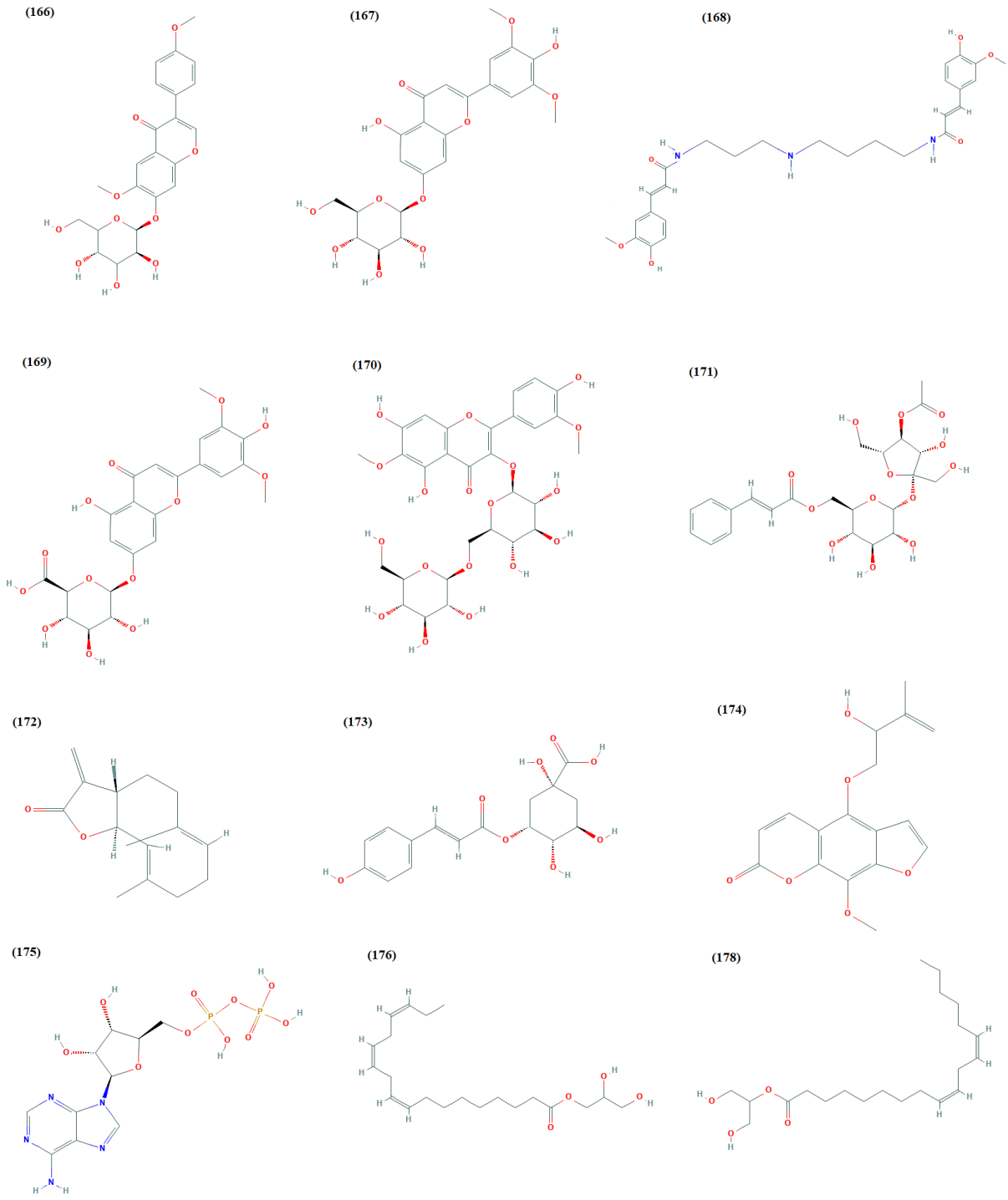

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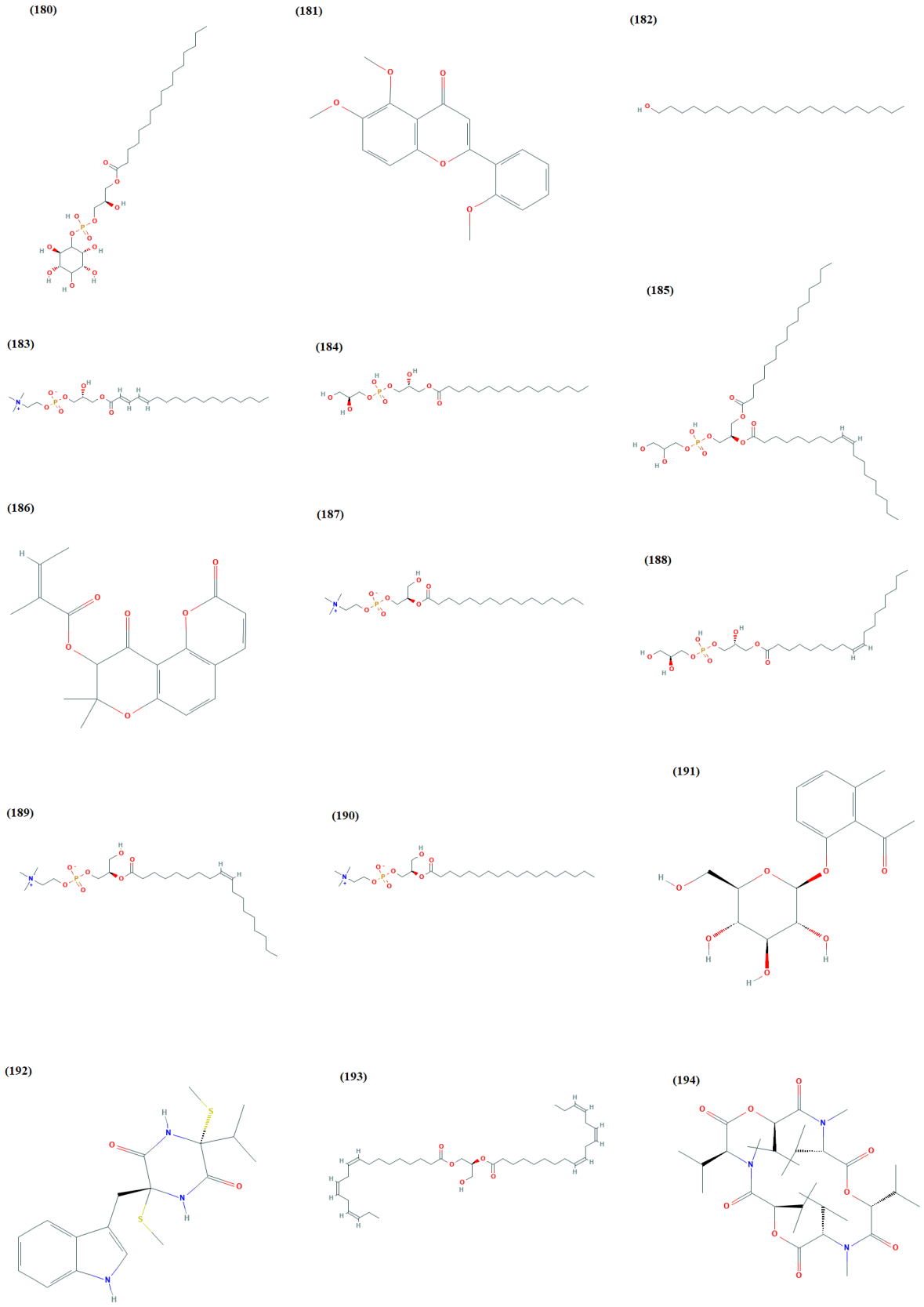

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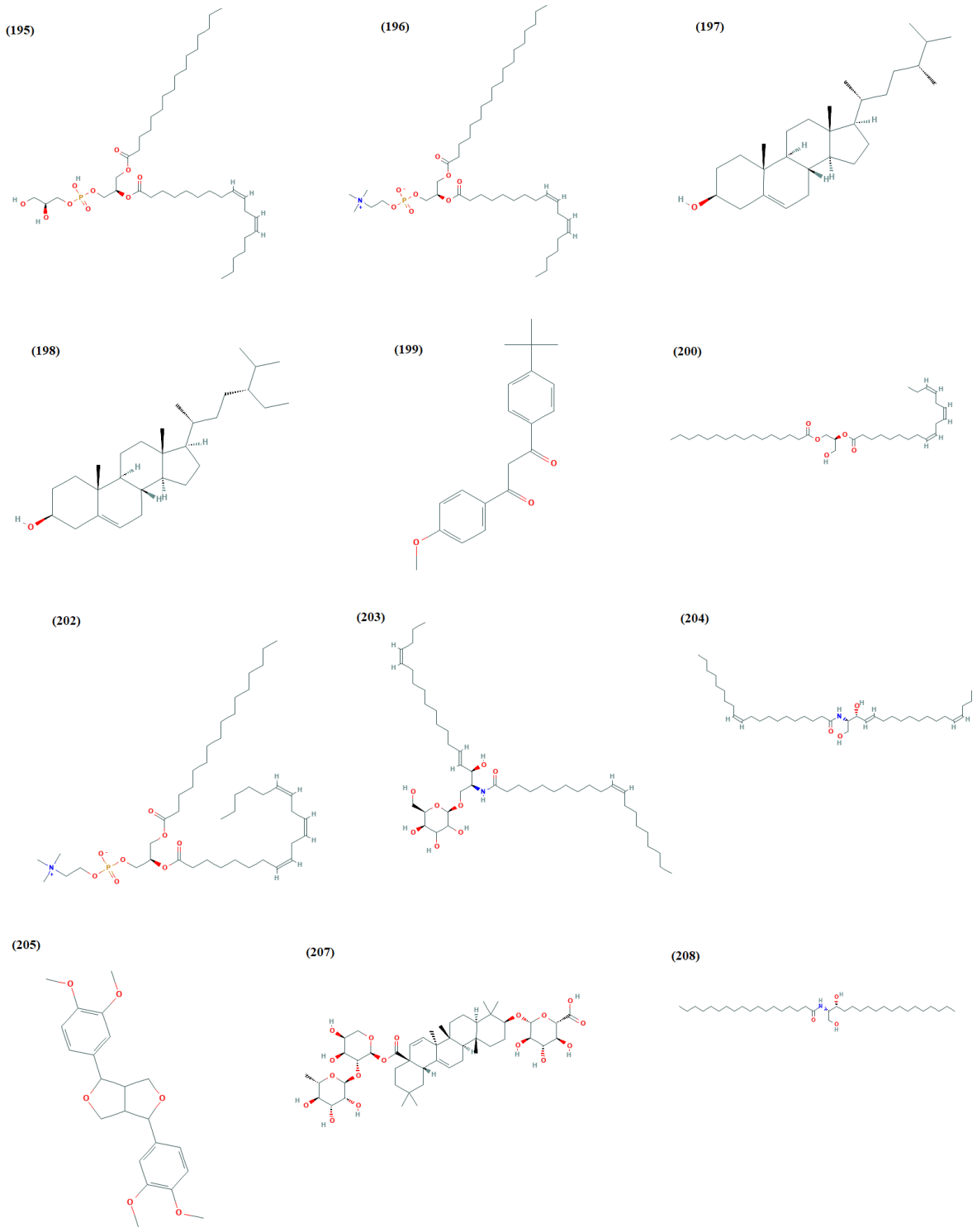

Figure 1.6 –continuation.

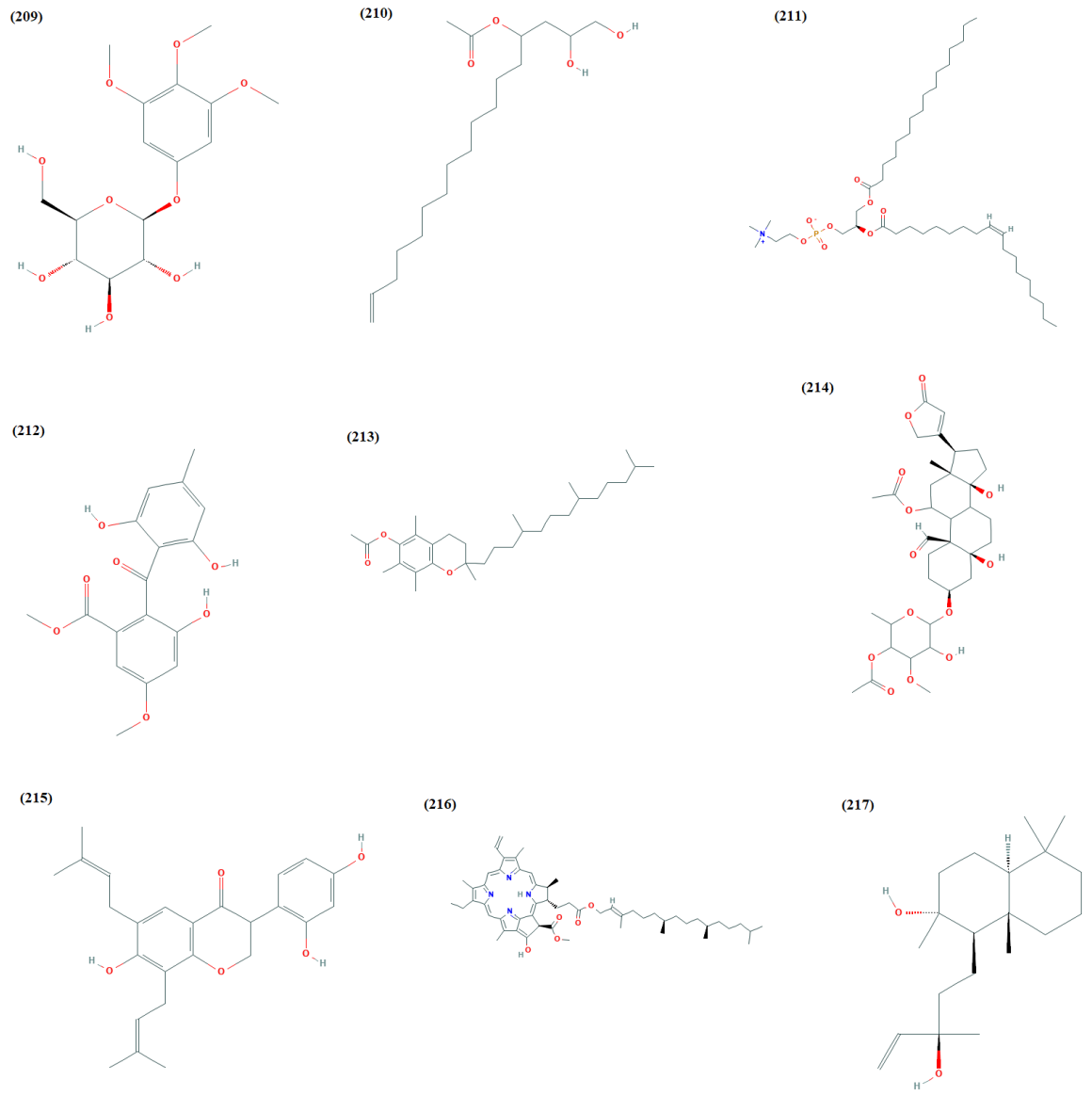

Figure 1.6 –continuation.

The lignan Matairesinol and the glycerophospholipid 2-octadecanoyl-sn-glycero-3-phosphocholine were detected in FC. The alkaloid Feruloyltyramine, the hydroxycinnamic acid derivative 3-*p*-coumaroylquinic acid, the glycerophospholipid 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, and the saponin Chiococcasaponin IV were detected only in MP.

Some of these other classes of metabolites have already been detected in other bamboo species, such as cardiac glycosides in *Guadua angustifolia* (Álvarez et al. 2015), saponins in *Bambusa ventricosa* and *Dendrocalamus strictus* (Joselin et al. 2014), alkaloids in *Oxytenanthera abyssinica* (Bartolhomew et al. 2013) and *Bambusa textilis* (Silva et al. 2012), among others. Many of the compounds detected in this study have never been described for bamboo before.

Therefore, it was observed that for the six analyzed species, phenolic compounds were the secondary metabolites class that presented the greatest diversity of substances, especially the flavonoids as seen in the heat map below (Figure 1.7).

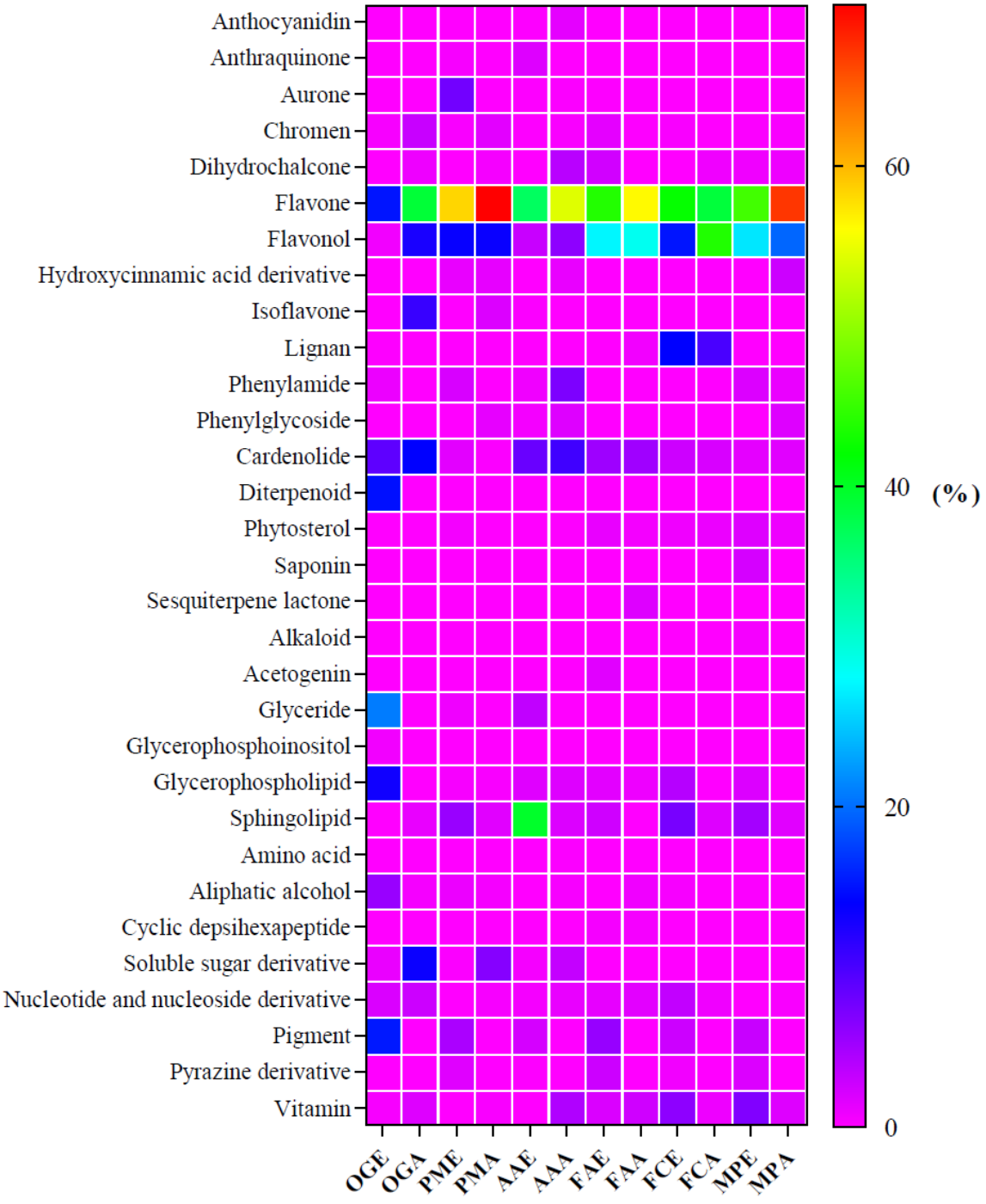

Figure 1.7 – Heat map of the relative percentage of the constituents present in hydroethanol **(E)** and aqueous **(A)** extracts analyzed by HPLC-ESIMS/MS in six Brazilian bamboo species. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata*; **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora*.

And as expected, the flavone class was the most expressive in the six study species, and interestingly, all species showed high levels of flavonols, something less reported in the literature for bamboo species (Figure 1.7).

Phenolic substances play important ecological functions for plants, as an example, flavonoids provide protection against UV radiation; lignin is responsible for plant mechanical support; anthocyanins confer colorations that attract pollinators and fruit dispersers. Therefore, some classes of the phenolic compounds are widely distributed in vegetables (Dewick 2009).

For humans, these molecules have enormous value, because many phenolic compounds have important biological activities, and may be used for different industry segments. Furthermore, bamboo extracts have different biological activities, many of which are attributed to the presence of phenolic substances.

To observe possible correlations between chemical composition and bamboo species a principal component analysis (PCA) was carried out. For that, relative percentage of some of metabolites classes analyzed by GC-MS and HPLC-ESIMS/MS were used. For phenolic compounds were considered chromenes, dihydrochalcones, flavones, flavonols, and phenolic acids. For terpenoids, phytosterols, cardenolides, and diterpenoids were used. Besides that, some other metabolites classes, as polyunsaturated and saturated fatty acids, soluble sugars and their derivatives, glycerides, and vitamin were considered in this analysis (Table 1.4).

PCA explained 84% of data variability within the first two axes (Figure 1.8). It was possible to observe the formation of two groups. Group 1, placed on the negative side of axis 1, is formed by FA, FC, and MP. Group 2 placed on the positive side of axis 1, is formed by AA and OG.

The highest levels of flavonols were determinant to separate the species of Group 1 on the negative side of axis 1 and 2. While the levels of soluble sugars and derivatives were determinant to separate AA on the positive side of axis 2; the levels of cardenolides separated OG on the negative side of axis 2 (Figure 1.8).

In summary, PCA showed that woody species have high levels of flavonols and this may be one of the chemical characteristics that separate them from herbaceous bamboo. PM, on the other hand, an herbaceous species showed high levels of flavones, a characteristic that was not shared by the other herbaceous species in this study.

Axis	Eigenvalue	% variance
1	980.522	61.968
2	354.738	22.419
3	200.086	12.645
4	333.746	21.092
5	135.826	0.85841

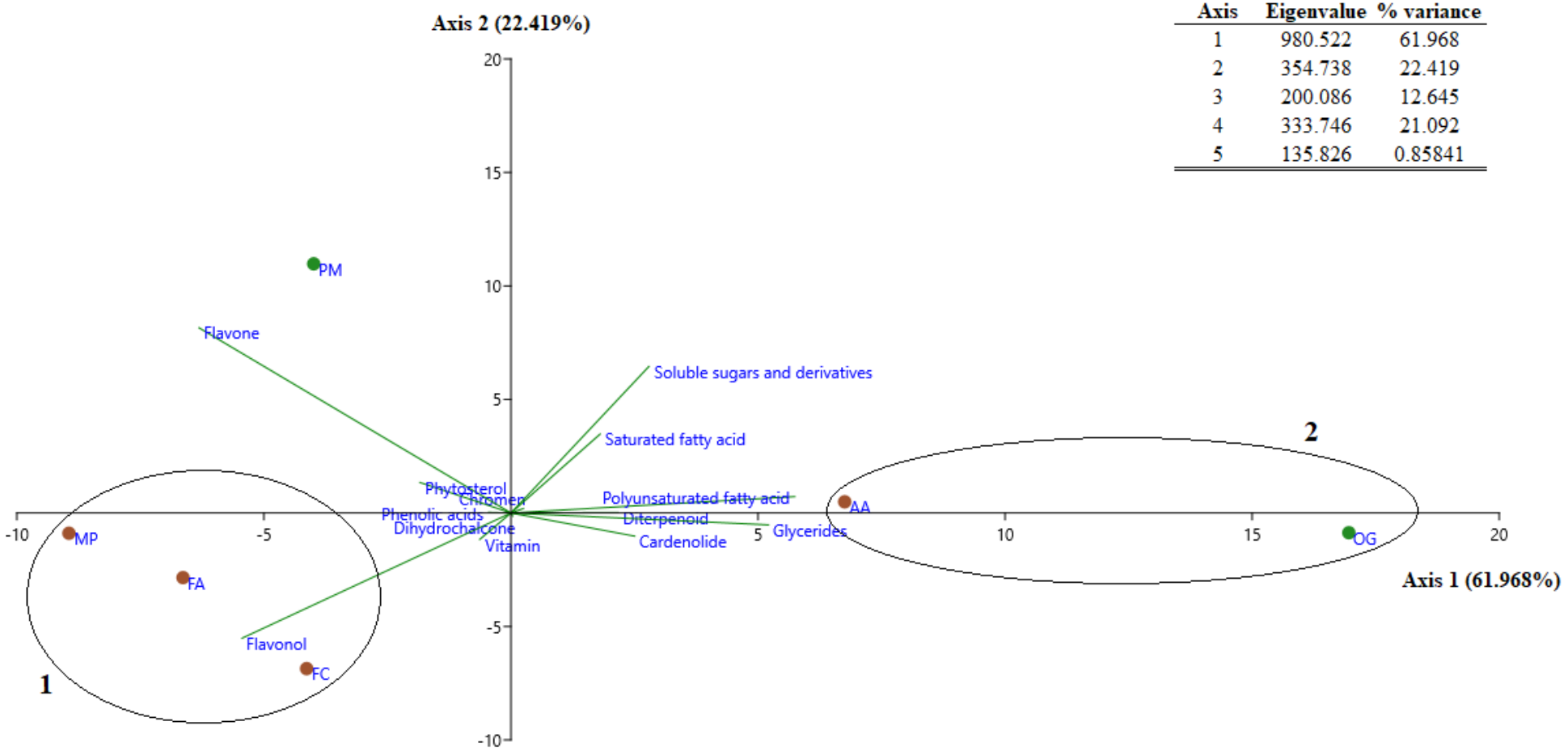

Figure 1.8 – Principal component analysis (PCA) using relative percentage of 13 classes of metabolites analyzed by GC-MS and HPLC-ESIMS/MS in six Brazilian bamboo species. Green symbols (herbaceous species): **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*. Brown symbols (woody species): **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.*

Table 1.4 – Correlation coefficients between the variables used in the principal component analysis (PCA).

Variables	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Cardenolide	0.200	-0.082	-0.095	0.190	0.543
Chromen	0.020	0.016	0.046	-0.001	0.093
Dihydrochalcone	0.003	-0.011	-0.065	0.060	0.091
Diterpenoid	0.174	0.002	0.130	-0.277	0.270
Flavone	-0.506	0.653	-0.276	0.035	0.192
Flavonol	-0.436	-0.442	0.434	0.177	0.036
Glycerides	0.417	-0.042	-0.506	0.121	-0.414
Phenolic acids	-0.036	0.002	-0.049	-0.076	0.139
Phytosterol	-0.148	0.108	-0.243	-0.042	0.257
Polyunsaturated fatty acid	0.460	0.058	0.122	-0.027	0.506
Saturated fatty acid	0.144	0.278	0.249	0.830	-0.035
Soluble sugars and derivatives	0.224	0.517	0.552	-0.320	-0.243
Vitamin	-0.051	-0.093	-0.063	-0.191	0.055

However, the presence of *A. aristulata* grouped together with *O. glaberrima* may infer a phylogenetically closer relationship between this woody species and the herbaceous species, when compared to the other woody species in this study. As we can see in the hierarchical analysis using the same 13 parameters analyzed in the PCA analysis (Figure 1.9).

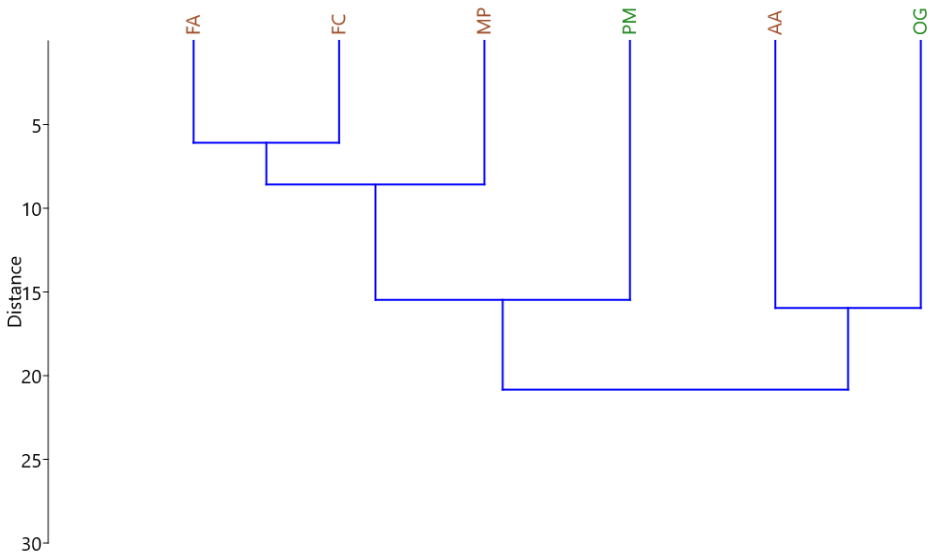

Figure 1.9 – Hierarchical analysis using relative percentage of 13 classes of metabolites analyzed by GC-MS and HPLC-ESIMS/MS in six Brazilian bamboo species. Green (herbaceous species): **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*. Brown (woody species): **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.*

According to Soreng et al. (2017) the Olyreae tribe is subdivided into 3 subtribes, among them are Olyrinae, which includes the two genera of herbaceous bamboo used in the present study, *Olyra* and *Parodiolyra*. Bambuseae tribe, on the other hand, is subdivided into 11 subtribes; all woody bamboo genera used in this study belong to Arthrostylidiinae subtribe.

Phylogenetic analyses suggested that *Parodiolyra* and *Olyra* are not monophyletic groups. *Parodiolyra* is paraphyletic to *Raddiella*; and *Olyra* is also paraphyletic with species of *Lithachne*, *Arberella*, and *Cryptochloa* (Oliveira et al. 2014).

Olyra was the first genus described for Olyreae and many of the current genera in this tribe were described based on species segregated from it. Nevertheless, there is great heterogeneity in the concept of species currently accepted in *Olyra*; Judziewicz et al. (1999) already indicated that this genus is probably polyphyletic. *Olyra* is also one of the few genera in the tribe that displays variation in the reproductive characters, its taxonomy being based primarily on differences in the structure of the female flower.

Oliveira et al. (2019) corroborate the paraphyly of *Parodiolyra*, with *P. micrantha* sister to a clade including the remaining *Parodiolyra* and *Raddiella*. Thus, based on phylogenetic and morphological evidence, the authors recircumscribe *Parodiolyra*, transferring *P. micrantha* and *P. colombiensis* to a new genus *Taquara*; described in their study.

The hierarchical analysis of this study reinforces the heterogeneity of OG and PM, although they are species belonging to the same subtribe, they are chemically different, corroborating their phylogenetic complexity.

Tyrrell et al. (2012) published the first multi-locus chloroplast phylogeny of Arthrostylidiinae, they sampled 51 taxa chosen to span the range of taxonomic diversity and morphology, and analyzed a combined chloroplast DNA dataset with six chloroplast regions. The authors observed a phylogenetic proximity between the species of *Filgueirasia* and *Merostachys*, they grouped them in the same clade.

In the hierarchical analysis carried out in the present study, it was also observed a chemical proximity between the species of *Filgueirasia* with the studied species of *Merostachys* (Figure 1.9). In general, we can infer that the chemical parameters analyzed in this study corroborate the phylogeny proposed for these bamboo genera.

1.4. Conclusions

The Brazilian bamboo species showed a great diversity of constituents. Although these metabolites have already been reported in other plant species, including in Asian bamboo species, it is the first time that they have been reported for these six Brazilian bamboo species.

Moreover, many classes of compounds that were detected in this study had never been described for bamboos before. We can infer that although there are several works of chemical characterization for Asian bamboo, many of them focused on phenolic substances, neglecting other classes of secondary metabolites, as terpenoids, alkaloids, phytosterols, and others. Many of these metabolites have potential for application in the pharmaceutical industry. Furthermore, some of the detected substances in this study have no reports in the literature regarding their biological activity.

Otherwise, Brazilian bamboo leaves presented important metabolites of nutritional value, such as amino acids, fatty acids, and vitamins, which could be of great interest to the food industry, with potential application as food additives, or even as nutraceutical.

Chapter 2

Screening for bioactive extracts from Brazilian bamboo species with an effect on cognition and memory

2.1. Introduction

The human brain is known to regulate several processes within the central nervous system or in the periphery, and it is considered the most complex organ in the human body. Cognition refers to mental processes related to obtaining and using the information to guide behavior such as learning, memory, attention, language, motor skills, perception, and executive function (Bakoyiannis et al. 2019).

Several types of mental illness attributed to aging are collectively named Dementia. This denomination is used for several progressive degenerative brain syndromes that affect memory, thinking, cognition, behavior, and emotion. There are over 100 forms of dementia. The most well-known form of dementia is Alzheimer's disease, which accounts for 50-60% of all dementia's cases. Other forms of dementia include vascular dementia, dementia with Lewy bodies, and frontotemporal dementia (ADI 2019).

In 2010, more than 135 million people were affected by some form of dementia. The global economic impact of Alzheimer's disease reaches 600 billion dollars a year, which is greater than important chronic diseases such as heart disease and cancer. The World Health Organization (WHO) recently declared dementia as a "public health priority" which should be on every public health agenda in the world (Langa 2015).

The most common signs between all types of dementia are memory loss, and the loss of practical abilities, which can lead to withdrawal from work or social activities (ADI 2019).

Cognitive impairment and memory loss are associated with reduced rates of acetylcholine (ACh) in the synaptic process, decreasing cortical cholinergic neurotransmission, and other neurotransmitters (Viegas Junior et al. 2004). ACh is a major signaling molecule in memory functions facilitated by the cholinergic system (Sikazwe et al 2017). Therefore, one of the main treatments in cases of dementia is to restore the cholinergic system using drugs capable of inhibiting the unrestrained action of the cholinesterase enzymes (ADI 2019).

Cholinesterases are a family of enzymes that catalyze the hydrolysis of acetylcholine to choline and acetate, in a process essential for restoring the neuronal system. They are divided into two types: acetylcholinesterase and butyrylcholinesterase (Pohanka 2011).

The principal biological role of acetylcholinesterase (AChE) is the termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the acetylcholine (ACh) (Dvir et al.

2010). Butyrylcholinesterase (BuChE), also known as pseudocholinesterase or nonspecific cholinesterase, catalyzes the hydrolysis of esters of choline, and is expressed in distinct populations of neurons, is a co-regulator of cholinergic neurotransmission and seems to be involved in some aspects of the development of the nervous system (Darvesh et al. 2003).

The search for new drugs for the treatment of any disease involves several steps, from *in vitro* assays to clinical trials in humans. At the screening process of new substances with the potential to restore the cholinergic system, a widely used *in vitro* approach is the colorimetric test developed by Ellman et al. (1961). It is a fast and sensitive alternative assay that uses cholinesterase enzymes to detect and select samples with anticholinesterase action (Ellman et al. 1961).

In addition to the therapeutic approach with cholinesterase inhibitors, another proposal that has been raised for the treatment of cognitive impairment is the use of antioxidants in combination with drugs or phytotherapeutics that target other pharmacological mechanisms (Williams et al. 2011). Thus, trials that evaluate antioxidant capacity may be great allies in the search for new bioactive substances against cognitive and memory losses.

Concerning the *in vivo* approach, zebrafish (*Danio rerio*) has become a powerful tool in neuroscience research. This species has their genome well-characterized and conserved, with over 70% of zebrafish genes sharing a high degree of similarity with their mammalian orthologs, furthermore, a small body size, ease of experimental manipulations, and rich behavioral repertoire are advantages for its use in experimentation (Shams et al. 2018; Fontana et al. 2018).

Natural products have played an important role in ancient traditional medicine systems, and are still in common use today. Plants are the best source for new drug scafolds because they have significant chemical structural diversity and offer a wide range of new pharmacophores. Although, there are about less than 1% of the land plants that have been explored in-depth for their phytochemistry or pharmacological potential (Rehman et al. 2019).

According to World Health Organization (WHO), 75% of the world population still depends on plant-based traditional medicines for primary health care, which involves the use of plant extracts or their bioactive secondary metabolites (Rehman et al. 2019).

Some phytoconstituents may improve memory as well as cognitive functions, and potentially suppress neurodegeneration of the brain. Neuroprotection refers to the mechanisms and strategies employed to defend the central nervous system against injury due to both acute and chronic neurodegenerative disorders (Rehman et al. 2019).

Many natural products have shown a neuroprotective effect against mental disorders (Essa et al. 2015). In this sense, several examples of biodiversity have been studied as a result of their

popular use, through ethnobotanical surveys. One of the most widespread examples of phytomedicine is the Ginkgo extracts. *Ginkgo biloba* (Ginkgoaceae) is used for centuries in traditional Chinese medicine to improve alertness. Currently, ginkgo extracts have been widely used specifically to increase cognitive function, being used in Europe for the treatment of several types of dementia (Gold et al. 2002).

Many of the protective effects of the central nervous system associated with the chronic use of Ginkgo extracts are related to the presence of terpene and flavonoid constituents with antioxidant and anti-inflammatory properties (Gold et al. 2002). Therefore, it is not surprising that plants that produce some positive effects on cognition have high levels of antioxidant substances, such as ginkgo.

There are reports in the literature on the traditional use of leaves of some Asian bamboo species for the treatment of mental illness. For example, Honfo et al. (2015) runed an ethnobotanical survey in a rural district of Benin, West Africa; 15% of the informants reported the use of *Oxytenanthera abyssinica*, *Bambusa vulgaris*, and *Dendrocalamus asper* leaves for treating nervous system disorder, as well as for memory issues.

Considering that Asian species are used in traditional medicine for the treatment of mental disorders, and most of their biological activities are attributed to the presence of phenolic substances, this study hypothesized that secondary metabolites presented in the Brazilian bamboo extracts have biological potential against diseases that affect cognition and memory.

There are different biological activities attributed to bamboo species and some of the traditional uses reported by bamboo have been tested, but there is a lack of knowledge about the benefits of bamboos against mental illness. In the last 11 years, only one study reported the anticholinesterase potential of a substance extracted from a bamboo species (see *Introduction*, Table 3, page 29).

Therefore, the present study aimed to analyze the potential of Brazilian bamboo extracts in the inhibition of two classes of cholinesterase enzymes, and analyze the antioxidant potential of these extracts. It was also analyzed the toxicological effect of the aqueous extracts on the morphology, locomotion and behavior of zebrafish larvae; and the potential of these extracts in improving the cognitive and memory functions in adults of zebrafish.

2.2. Material and Methods

2.2.1. Plant material and extracts preparations

This study used six Brazilian bamboo species (see *Introduction*, page 49): *Aulonemia aristulata* **(AA),** *Filgueirasia arenicola* **(FA)**, *Filgueirasia cannavieira* **(FC)**, *Merostachys pluriflora* **(MP)**, *Olyra glaberrima* **(OG)**, and *Parodiolyra micrantha* **(PM)**. For biological assays, leaves were submitted to maceration and infusion extraction, as described in *Chapter 1*, pages 52 and 53.

2.2.2. *In vitro* biological assays

Aqueous, hydroethanol, and hexane extracts from all bamboo species were tested regarding their antioxidant and anticholinesterase activities. Leaf samples were composed by a poll of individuals characterizing a single composite sample for each species. Antioxidant and Anticholinesterase analyzes were performed in methodological triplicate, and the measures were done using Synergy H1 equipment (BioTek, Inc.).

2.2.2.1. Anticholinesterases activities

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) activities were measured according to Mathew and Subramanian (2014) based on Ellman's method (Ellman et al. 1961), with some modifications. The enzymes AChE and BChE hydrolyze the substrate acetylthiocholine and butyrylthiocoline, respectively. This reaction produces thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercapto thiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm.

Tris–HCl (50 mM, pH 8.0) was used as a buffer throughout the experiment unless otherwise stated. AChE used in the assay was from electric eel (type VI-S lyophilized powder, 987 U/mg solid) and the same for BChE (lyophilized powder, 140 U/mg solid). The enzymes stock solutions (AChE 493.5 U/mL and BChE 140 U/mL) were kept at -80ºC. The further enzyme-dilution was done in 0.1% BSA (bovine serum albumin) in Tris–HCl buffer. DTNB was dissolved in the buffer containing 0.1 M NaCl and 0.02 M $MgCl_2$. Acetylthiocholine (ATCI) and Butyrylthiocoline (BTCI), both at 18 mM, were dissolved in ultrapure water.

In the 96-well plates were mixed 100 µL of 5 mM DTNB, 20 µL of 0.26 U/mL of AChE or 0.30 U/mL of BChE, 40 µL of Tris–HCl buffer, 20 µL of each extract in various concentrations (2.0, 1.0, 0.5, 0.25, 0.12, 0.06, and 0.03 mg/mL), also dissolved in Tris–HCl buffer. After mixing, the plate was incubated for 15 min (25ºC) and then the absorbance was measured at 412 nm.

The enzymatic reaction was initiated by the addition of 20 μL of 18 mM ATCI or BTCI and the hydrolysis was monitored by reading the absorbance every 5 min for 20 min. Neostigmine was used as a positive control. Negative control with buffer was run with each assay. All the reactions were performed in triplicate. The percentage of inhibition was calculated as follows:

% Inhibition = 100 − [(Absorbance of sample/Absorbance of negative control)] × 100.

The IC_{50} were calculated by using a regression equation between the concentration and the inhibition percentage of each sample and were expressed in μg/mL.

2.2.2.2. Determination of DPPH radical scavenging capacity

DPPH radical scavenging activity was determined according to Furlan et al. (2015). DPPH solution in methanol (0.20 mM) was freshly prepared and 200 μL was mixed with 20 μL of each sample (12.5 to 1.000 μg/mL) in 10% DMSO. The reaction mixtures were incubated for 20 min in the dark, and the decrease in absorbance was measured at 515 nm. As positive control, a methanol solution of Trolox (12.5 to 200 μg/mL) was used. All measurements were performed in triplicate and a negative control (methanol and 10% DMSO) was run with each assay. The percentage of free radical scavenging was calculated using the formula:

% = 100 − [(Absorbance of sample/Absorbance of negative control)] × 100

The EC_{50} were calculated by using a regression equation between the concentration and the antioxidant percentage of each sample and were expressed in μg/mL.

2.2.2.3. Determination of oxygen radical absorbance capacity (ORAC)

ORAC assay was performed according to Santos et al. (2016). Sodium fluorescein was dissolved in phosphate buffer solution (PBS) (75 mM, pH 7.0) to obtain a stock solution of 4.0 μM. The fluorescein working solution (48 nM) was freshly prepared in PBS, and 150 μL were mixed with 25 μL of each sample (in PBS) at different concentrations (0.97 to 125 μg/mL). The reaction mixtures were incubated for 30 min at 37°C and a 25 μL of 75-mM AAPH solution was added. As a positive control, a PBS Trolox solution was plotted (6.25 to 50 mM). All measurements were performed in triplicate. A negative control with PBS was run with each assay. The fluorescence (excitation = 485 nm; emission = 528 nm) was registered 120 times with a delay of 60 s between repeats.

The antioxidant capacity was based on the calculation of the area under the curve (AUC), using the following formula: $(AUC) = [1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + \ldots f_i/f_0]$. Where f_0 is the initial

fluorescence reading at 0 min and f_1 is the fluorescence reading at time 1. The net AUC was obtained by subtracting the AUC of the blank from the AUC of the sample.

The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net AUC, and the results were expressed as Trolox equivalents (TE, μMol Trolox/g extract). The percentage of ORAC was calculated using the formula:

$$\% = [(\text{Net AUC of sample} \times 100)/\text{Net AUC of positive control}]$$

The EC_{50} were calculated by using a regression equation between the concentration and the antioxidant percentage of each sample and were expressed in μg/mL.

2.2.3. *In vivo* biological assays

For all *in vivo* biological assays were used only the aqueous extracts from all studied species, at four different concentrations (1.5, 1.0, 0.5, and 0.1 mg/mL in water). These procedures were conducted at the Pontifícia Universidade Católica do Rio Grande do Sul (PUC-RS), with the collaboration of Drª Carla Denise Bonan and Drª Stefani Altenhofen. All protocols were approved by the Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul (9413, CEUA/PUCRS).

The extracts concentrations were chosen based on previoslly *in vitro* assays using bamboo extracts.

2.2.3.1. Animal maintenance

Embryos and larvae (0-5 days post-fertilization (dpf)) and adult stage animals (12-18 months, 0.3-0.6 g) of wild-type *Danio rerio*, from the AB background were obtained from PUCRS breeding colony. A total of 2400 embryos and 360 adult animals were used for the development of this study. Sample sizes vary slightly among variables due to technical problems with the behavior analysis software and mortalities. The animals were maintained in recirculating systems (Zebtec, Tecniplast, Italy) with reverse osmosis filtered water equilibrated to reach the species standard temperature (28°C ± 2°C), pH (7.0 and 7.5), and ammonia, nitrite, nitrate, and chloride levels. Animals were subjected to light/dark cycle of 14/10 hours, respectively. Animals received paramecium between 6 and 14 dpf and after 14 dpf, they received commercial flakes (TetraMin Tropical Flake Fish®) three times a day, supplemented with brine shrimp (Westerfield 2000).

For breeding, female and males (1:2) were placed in breeding tanks overnight separated by a transparent barrier that was removed after the lights went on the following morning. The fertilized eggs retained in the fitted tank bottom were collected, sanitized and immediately subjected to the treatment. The embryos were maintained for up to 5 days post-fertilization (dpf)

at a density of one larva per 7 mL in Petri dishes in a biochemical oxygen demand (BOD) incubator. They were immediately transferred to a tank with a density of one larva per 60 mL. When the animals reached 30 dpf, they were maintained at a density of one animal per 200 mL until adulthood.

Water used in the experiments was obtained from a reverse osmosis apparatus (18 MOhm/cm) and was reconstituted with marine salt (Crystal Sea™, Marinemix, Baltimore, USA) at 0.4 ppt. The total organic carbon concentration was 0.33 mg/L. The total alkalinity (as CO_3^{2-}) was 0.030 mEq/L. During fish maintenance, water parameters were monitored daily and maintained in the following ranges: pH: 6.5 to 7.5, conductivity: 400 to 600 μS, ammonium concentration: < 0.004 ppm, and temperature: 25 to 28°C.

2.2.3.2. Survival rate

A total of 400 embryos were used for survival analysis for each extract. Embryos were placed in Petri dishes (20 embryos per dish) and subjected to bamboo extracts treatment at doses of 0 (control group), 0.1, 0.5, 1.0, and 1.5 mg/mL for five days (from 1-hour post fertilization (hpf) to 5 dpf). Animals were monitored daily for survival rate (n = 80, per group) using an inverted stereomicroscope (Nikon, Melville, EUA). After this treatment, the animals were subjected to experimental tests. The hatching rate was also monitored; however, there was no difference between these two groups (data not shown).

2.2.3.3. Morphological measures

The potential toxicity of bamboo extracts was estimated after the treatment by monitoring morphological defects in 5 dpf larvae under a stereomicroscope. The animals used for morphological evaluation were selected from different plates, using the same animals for survival analysis. Body length (μm), ocular distance (μm), and surface area of the eyes (μm^2) were evaluated (n = 30, per group) using NIS-Elements D software for Windows 3.2 (Nikon Instruments Inc., Melville, USA). Body length was defined as the distance from the larval mouth to the pigmented tip of the tail, the ocular distance was evaluated by the distance between the inner edge of the two eyes (similar to the inner intercantal distance in humans), and the size of the eyes was determined by measuring the surface area of the eyes (Altenhofen et al. 2017; Nabinger et al. 2018).

2.2.3.4. Behavioral analyses

2.2.3.4.1. Exploratory behavior

The exploratory behavior of the larvae exposed to bamboo extracts was based on Creton (2009) and Nery et al. (2017), and evaluated at 5 dpf (n = 18-36, per group). The animals used for exploratory behavior evaluation were selected from different plates, using the same animals for survival analysis. The experiments were performed in a temperature-controlled room (27 ± 2°C) between 13:00 and 17:00. The behavior of the animals was recorded in a video during 5 minutes after the 60 seconds-habituation period and analyzed using EthoVision XT software (version 11.5, Noldus) (Figure 2.1).

Each larva was individually placed in a cell culture 24-well plate containing 2 mL of water per well, in a designed protocol that virtually divided each 15 mm diameter well in an inside area (7.5 mm diameter) and an outside area. The locomotor parameters evaluated were the distance traveled (cm), velocity (s), time mobile (s), and absolute turn angle (º). The anxiety behavior was also measured, as well as the time spent outside area (s). Each well position (outside *vs*. inside area) was considered an index of anxiety.

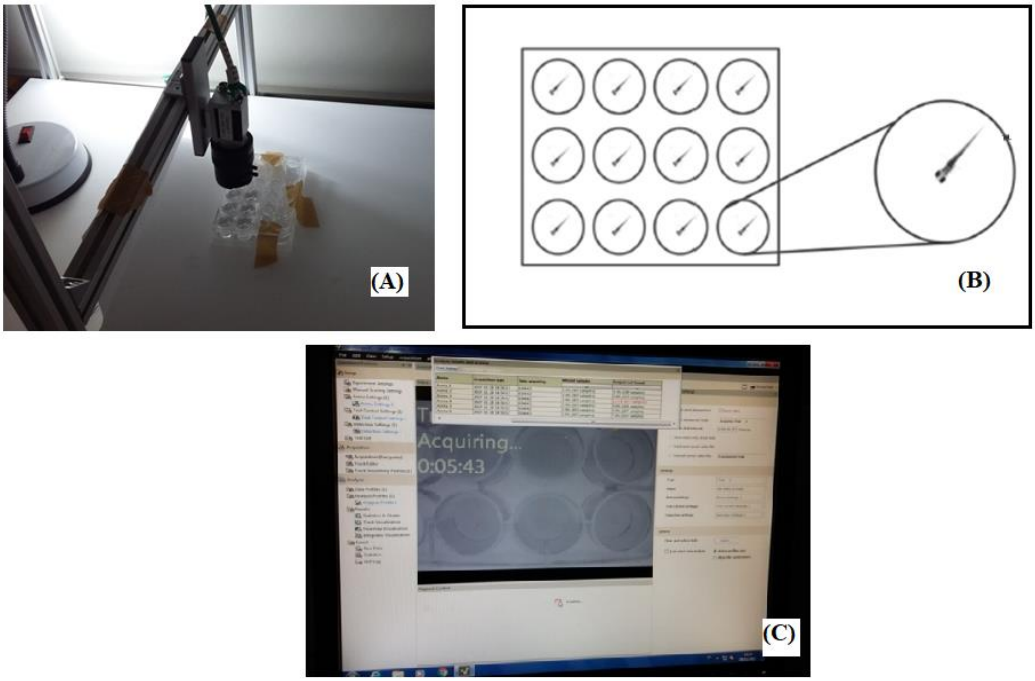

Figure 2.1 – Exploratory behavior apparatus. **(A)** recording of the larvae behavior; **(B)** representation of the larvae arranged on the plate; **(C)** recording of larvae exploratory behavior on the computer. Photos: J. Gagliano (2017); Figure: Siebel et al. (2015).

This task exploits the natural tendency of zebrafish to spend most of the time at the outside area when introduced to a novel environment. Then, the animals gradually extend the swimming range, over a period of minutes, to include the inside portion of the test well. A longer time spent in the outside area and less time spent in the inside of the well indicates increased anxiety for the larvae (Colwill and Creton 2011).

2.2.3.4.2. Avoidance behavior

After the exploratory evaluation of larvae (5 dpf) from bamboo extracts exposure, the animals were placed in 6-well plate (five larvae per well, n = 20-40, per group) over a LCD monitor for measuring cognitive ability and avoidance responses to a visual stimulus (a 1.35 cm diameter red bouncing ball) (Pelkowski et al. 2011) during a 5 min-session following 2 minutes of acclimation (Figure 2.2). The animals used for avoidance behavior evaluation were selected from different plates, using the same animals for survival analysis. The red bouncing ball traveled from left to right over a straight 2 cm trajectory on half of the well area (stimuli area), which animals avoided by swimming to the other non-stimuli half of the well. The number of larvae on the non-stimuli area during the 5 minutes session was considered indicative of their cognitive ability.

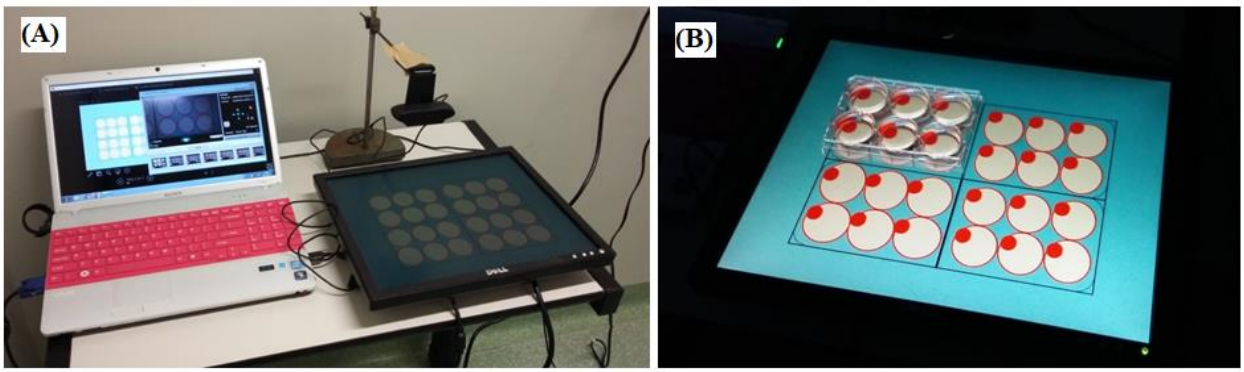

Figure 2.2 – Avoidance behaviour apparatus. **(A)** overview of the LCD screen and the computer where the larvae aversive behavior is recorded. **(B)** view of the LCD screen where the plates with the larvae are placed. Photos: J. Gagliano (2017).

2.2.3.4.3. Inhibitory avoidance task

Adults zebrafish (n = 60, per bamboo extract) were anesthetized by immersion in 0.1 g/L tricaine solution (ethyl 3-aminobenzoate methanesulfonate salt) before bamboo extracts or saline solution administration. Bamboo extracts, at doses of 0.1 and 0.5 mg/kg, were administered via intraperitoneal (i.p.) injection in a volume of 10 μL using a 3/10-mL U100 BD Ultra-Fine™

Short Insulin Syringe 8 mm (5/16”) × 31G Short Needle (Becton Dickinson and Company, New Jersey, USA) (Kinkel et al. 2010).

Drug doses and administration routes were chosen and adjusted based on toxicity presented in larval stage animals. After treatment, animals were placed in a separate tank with aerated and unchlorinated tap water to recover from the anesthesia. Bamboo extracts and saline solution (used as control group) were injected 2 h before the beginning of each experiment. One hour prior to the start of the behavioral assay, animals were transferred to a tank containing 200 μM scopolamine solution ((−)-Scopolamine hydrobromide trihydrate), dissolved in system water. Animals that did not receive scopolamine were also transferred to another tank with water to control for handling effects (Bortolotto et al. 2015; Kim et al. 2010).

To evaluate the potential protective effect of bamboo extracts on scopolamine-induced memory impairment, the inhibitory avoidance test was performed after scopolamine exposure (n = 8-10, per group) between 9:00 a.m. – 12 p.m. (Blank et al. 2009; Nabinger et al. 2018). There were two sessions, training and test, with a 24 h interval between them.

In each session, animals were placed individually in an experimental tank (18 cm long x 9 cm wide x 7 cm high) with water, divided by a guillotine door into two compartments of equal size, one black (right side) and one white (left side). During the training session, the animal was placed in the white compartment with the door closed for one minute for habituation and environment recognition. After this period, the division was lifted. Once the animal crossed into the black side of the tank, the guillotine door was closed again and using two electrodes attached to an 8.8 V stimulator, a final shock pulse of 3 ± 0.2 V AC (intensity measured between electrodes and the center of the dark compartment) was administered for three seconds (Figure 2.3).

Each animal was removed from the apparatus and returned to its housing water-filled tank for 24 h until the test session, which consisted of the same protocol as the training session, but without the electric shock. The latency to enter the black compartment during each session was measured and the expected increase in the test session was used as an index of memory retention. A 60 and 180 s ceiling were imposed on training and test session latency measurements, respectively.

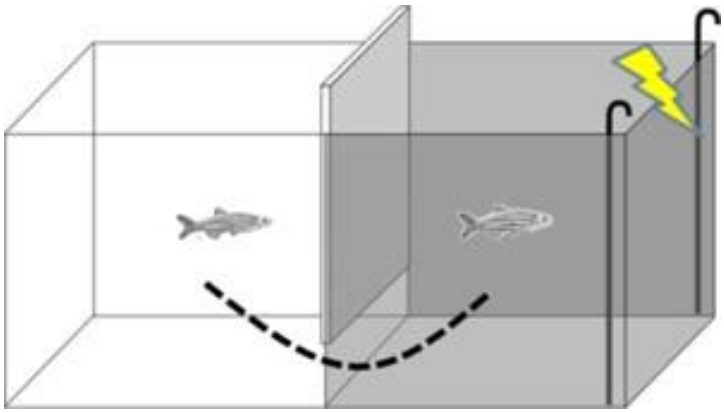

Figure 2.3 - Representation of the experimentation tank for the inhibitory avoidance test. Figure source: D. Nabinger.

2.2.4. Statistical analysis

Survival rate were analyzed by a Kaplan-Meier test. Data from morphological evaluation, exploratory, and avoidance behavior were compared using one-way ANOVA followed by a post-hoc Dunnet's test or a Kruskal-Wallis test followed by a post-hoc Dunn's test, depending on the normality of the data (assessed by the Kolmogorov-Smirnov test). Inhibitory avoidance training and test latencies within each group were compared using two-way ANOVA test. Latencies of multiple groups were compared by Mann-Whitney U or Student's *t* tests depending on normality of the data (assessed by the Shapiro-Wilk test). For all comparisons, the level of significance was defined as $p \leq 0.05$.

2.3. Results and discussion

2.3.1. *In vitro* biological assays

Aqueous, Hydroethanol and Hexane extracts from all studied species were evaluated for their antioxidant activity by DPPH radical scavenging activity, oxygen radical absorbance capacity (ORAC), and anticholinesterases activity using Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) enzymes.

2.3.1.1. Antioxidants

For antioxidant assays, results were expressed as effective concentration to reach 50% of the antioxidant activity ($EC_{50,}$ µg/mL). For DPPH and ORAC assays results were also expressed as mg/g of Trolox equivalent and µMol of Trolox equivalent, respectively (Table 2.1). For these analyses, samples of *Merostachys pluriflora* were not tested in this study, because they were previously described by Gagliano et al. 2018. All hydroethanol and aqueous extracts presented high *in vitro* antioxidant potential (Table 2.1).

Table 2.1 - *In vitro* antioxidant activitiy of extracts from bamboo leaves analyzed by DPPH and ORAC assays. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata*; **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora*. The final letter in each acronym of the bamboo species corresponds to the type of extract**: (E)** Hydroethanol extract, **(A)** Aqueous extract, **(H)** Hexane extract.

Samples	DPPH		ORAC	
	EC_{50} µg/mL	**mg/g TE**	**EC_{50} µg/mL**	**µMol/g TE**
OGA	325.23	26.86 ± 1.81	107.37	453.28 ± 47.00
PMA	118.85	74.90 ± 4.52	36.50	1311.92 ± 112.22
FAA	150.20	59.22 ± 2.13	38.77	1325.79 ± 104.84
FCA	171.03	51.77 ± 1.24	45.36	1114.49 ± 108.72
AAA	215.38	43.30 ± 0.85	70.77	828.04 ± 137.60
OGE	545.13	24.15 ± 3.92	78.87	674.01 ± 76.65
PME	102.37	84.88 ± 5.32	22.03	1583.30 ± 240.51
FAE	99.45	86.88 ± 2.90	30.66	1719.47 ± 209.50
FCE	115.93	76.41 ± 5.94	25.42	1775.09 ± 124.21
AAE	208.07	48.85 ± 0.71	50.56	999.00 ± 76.46
OGH	2370.05	12.98 ± 3.35	272.85	158.77 ± 21.68
PMH	664.14	20.51 ± 0.69	452.61	92.45 ± 12.61
FAH	2887.13	22.82 ± 0.36	727.16	63.52 ± 5.80
FCH	-	19.28 ± 0.64	2725.29	31.43 ± 7.70
AAH	-	12.04 ± 1.66	458.48	115.68 ± 16.04
Trolox	94.68	*	0.24	*

-: values without linear dose/response correlation.

*: not applied.

EC_{50}: Effective concentration to reach 50% of the antioxidant activity.

For ORAC assay, the majority of these extracts showed EC_{50} below 100 µg/mL. The values ranged from 22.03 µg/mL to 2725.29 µg/mL. For this assay, the most promising samples were PME and FCE with the lowest EC_{50} (22.03 µg/mL and 25.42 µg/mL, respectively) (Table 2.1). However, MP has been previously studied and the hydroethanol extract from its leaves showed EC_{50} smaller than the observed for these species, 5.79 µg/mL (Gagliano et al. 2018).

EC_{50} for DPPH assay ranged from 99.45 µg/mL in FAE to 2887.13 µg/mL in FAH. Similar to ORAC, in DPPH assay, aqueous and hydroethanol extracts showed the lowest EC_{50}. The hydroethanol extract of MP was also previously evaluated, presenting EC_{50} of 119.51 µg/mL (Gagliano et al. 2018).

Hexane extracts showed the lowest values of Trolox equivalent for both antioxidant assays. FAE (86.88 ± 2.90 mg/g TE) presented the highest content of Trolox equivalent in DPPH assay. For ORAC, the highest value was observed in FCE (1775.09 ± 124.21 µMol/g TE) (Table 2.1).

It was observed that the free radical scavenging activity of the bamboo extracts differed between the both applied methods. These differences, according to the methodologies, have already been reported by other authors. Tabart et al. (2009) evaluated the antioxidant capacity of some phenolic standards and some foods that have antioxidant compounds in their composition. The authors observed that most compounds showed significant differences in free radical scavenging activity according to the method used. Of the 25 tested compounds, only a few gave comparable activities in the various tests applied.

Despite the diversity of methods for assessing antioxidant capacity, there is no universal methodological procedure. Therefore, it is necessary to evaluate the antioxidant capacity by different tests, with different mechanisms of action (Oliveira et al. 2009). Therefore, the comparison of the antioxidant capacity between the different methods is not done in absolute values, since each method has its own scale. However, Huang et al. (2005) listed some important criteria to follow when chosing an assay, such as: use biologically relevant molecules; be technically simple; use a well-defined endpoint and chemical mechanism; have easily available instrumentation; have good repeatability and reproducibility; be adaptable for testing from hydrophilic to lipophilic antioxidants; and serve for *high-throughput* analyzes.

Although the two methods used in this study assess the free radical scavenging activity of a compound or an extract, these tests differ concerning to the reaction mechanism, the target reactive species, and the reaction conditions.

ORAC uses biologically relevant free radicals. In this assay, free radicals from the thermal decomposition of AAPH are generated at a constant rate throughout the test. ORAC assay is the only method that integrates both degree and time of antioxidant reaction (Tabart et al. 2009; Zulueta et al. 2009). DPPH molecule is a stable synthetic free radical that has a violet color, but when it is reduced by antioxidant compounds results in the formation of a pale-yellow hydrazine (Karadag et al. 2009).

Although both methods are widely used to evaluate the antioxidant capacity of compounds, extracts, and foods, both have their methodological limitations and the results must be carefully evaluated (Karadag et al. 2009).

As seen in the previous chapter, the species in this study showed a wide variety of phenolic substances in their composition (see *Chapter 1*, Table 1.3, page 74). Among the identified phenolic substances in the bamboo extracts, flavonoids are the major compounds. Flavonoids are the group of secondary metabolites with the highest occurrence in plants and their presence and abundance are correlated to different biological activities, including high antioxidant capacity (Rice-Evans et al. 1997).

The biological activities attributed to flavonoids are directly related to the different chemical structures that these substances may present (Rice-Evans et al. 1997; Kumar and Pandey 2013). The structural configuration, the substitution pattern, and the number of hydroxyl groups can directly influence flavonoids' antioxidant mechanism of action. The configuration of the hydroxyl groups in the B ring of a flavonoid is the most important feature for the elimination of reactive oxygen species, such as by donating a hydrogen and/or an electron to oxidant compounds, thus stabilizing them (Kumar and Pandey 2013).

Although all hydroethanol and aqueous extracts of the studied species have shown a great diversity of phenolic compounds in their composition, PME and PMA stood out with the greatest diversity of phenolic compounds among all analyzed species. PME and PMA showed 27 and 28 different phenolic compounds, respectively (see *Chapter 1*, Table 1.3, page 74). It was also observed that these two extracts showed low EC_{50}, for both antioxidant assays, meaning that these extracts had the highest antioxidant activity (Table 2.1).

Regarding the EC_{50} observed in the ORAC assay, all of them were below 100 µg/mL. According to Cos et al. (2006), samples composed by a mixture of constituents as extracts, and presenting EC_{50} below 100 µg/mL are considered promising for future research, as *in vivo* assays. Therefore, according to the methodologies used in this study, the analyzed bamboo species are potential candidates as sources of antioxidant compounds.

It was already expected that hexane extracts would have the less antioxidant capacity. This solvent prioritizes the extraction of lipophilic compounds, such as fatty acids, terpenoids, and alkanes (see *Chapter 1*, Table 1.2, page 65). As reported in the literature, polyphenols possess ideal structural chemistry for free radical-scavenging activities, being more effective antioxidants, but having a more lipophobic behavior (Rice-Evans et al. 1997).

The antioxidant capacity of bamboo extracts is widely studied mainly in Asian species. In the last decade, half of the studies of bamboo biological activities analyzed the antioxidant

potential of these species, and about 50% of these studies used DPPH as the major method of analysis (see *Introduction*, Table 3, page 29).

In a recent study with Brazilian bamboo species, Wroblewska et al. (2019) analyzed the antioxidant potential of hydroethanol extracts from leaves of 5 native species of bamboo. EC_{50} reported for DPPH ranged from 137.55 µg/mL to 260.17 µg/mL, values very similar to the observed in the present study for hydroethanol extracts (Table 2.1).

Wroblewska et al. (2019) also analyzed *Aulonemia aristulata* and *Merostachys pluriflora* observing EC_{50} of 260.17 µg/mL and 222.52 µg/mL, respectively. Similar values were observed for *Aulonemia aristulata* hydroethanol extract in this study (208.07 µg/mL), and it was also similar to the reproted by Gagliano et al. (2018) for *Merostachys pluriflora* hydroethanol extract (119.51 µg/mL).

The most studied bamboo species in the last decade belong to the genera *Phyllostachys*, *Bambusa,* and *Sasa*. These genera comprise species of great commercial interest, being used in the food industry as an antioxidant additive (Zhang et al. 2005), or the shoots are commonly consumed *in natura* because they are considered very nutritious and also a source of natural antioxidants as well (Nirmala et al. 2018).

EC_{50} reported in the DPPH assay for leaf methanol extracts from *Bambusa nutans* (123.45 µg/mL) and *Bambusa vulgaris* (262.90 µg/mL) (Tripathi et al. 2015) are also very similar to those observed in the present study for hydroethanol extracts from Brazilian bamboo species. However, leaf extracts from *Phyllostachys nigra* presented EC_{50} values ranging from 1590.0 µg/mL to 14640.0 µg/mL (Ma et al. 2020), values way-above of the ones observed for Brazilian species.

It is important to mention that the leaf extracts of some *Phyllostachys* species are used as food antioxidant additive in China (Zhang et al. 2005), and these commercialized extracts have already been shown to reduce the formation of acrylamide, a carcinogenic compound formed in the frying process of some foods (Zhang et al. 2007). Therefore, considering the low EC_{50} presented by the bamboo extracts analyzed in this study, Brazilian species could be better investigated as a potential natural food antioxidant additive and/or as a dietary supplement.

2.3.1.2. Anticholinesterases

For anticholinesterase assays, results were expressed as the concentration to reach 50% inhibition of the enzymes activity ($IC_{50,}$ µg/mL) (Table 2.2). All tested samples were able to inhibit the action of AChE enzyme, the lowest IC_{50} was observed for MPE (401.11 µg/mL). None of the tested samples inhibited the BChE activity.

The cholinergic function is important for the processes of learning and memory (Deutsch 1971). Therefore, one of the first approaches in the treatment of patients with cognitive and memory decline is the restoration of cholinergic processes, through drugs that can inhibit the action of cholinesterase enzymes. An anticholinesterase slows down the degradation of ACh, so the neurotransmitter spends more time in the crack synaptic, thus intensifying the cholinergic transmission (Araújo et al. 2016).

Table 2.2 - *In vitro* acetylcholinesterase activity of the extracts from bamboo leaves. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata*; **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora*. The individual letters correspond to the type of extract**: (E)** Hydroethanol extract, **(A)** Aqueous extract, **(H)** Hexane extract.

Species	Extracts - IC_{50} (µg/mL)		
	(A)	**(E)**	**(H)**
OG	696.63	1010.15	3496.24
PM	425.20	17406.81	2968.94
FA	873.26	2447.32	727.01
FC	609.44	568.75	3690.24
AA	2091.57	439.09	8286.20
MP	423.24	401.11	494.21
Neostigmine	0.02		

IC_{50}: Concentration to reach 50% inhibition.

Since the discovery of the Physostigmine, an alkaloid obtained from *Physostigma venenosum* L., the first known anticholinesterase agent, natural products have been investigated as a source of important molecules for the restoration of the cholinergic system (Araújo et al. 2016).

There are reports in the literature of the traditional use of bamboo leaf extracts for the treatment of mental disorders and to improve memory (Honfo et al. 2015). Although it is reported the use of these species in traditional medicine, few studies have been conducted so far to investigate the potential action of bamboo extracts on cognition and memory.

It is worth mentioning the study developed by Liu et al. (2015) which observed that bamboo leaf extract was able to improve the spatial learning ability of the dementia in rats. They also observed that the hippocampus of dementia model rats showed reduced levels of acetylcholine, epinephrine, norepinephrine, and dopamine, also showing increased activities of

acetylcholine esterase and monoamine oxidase. Animals treated with bamboo extract had significantly inhibition on the enzyme activity compared with untreated dementia rats. The authors suggested that bamboo extracts might be a potential drug in treating impairment of spatial memory in dementia rats by regulating the central neurotransmitter function.

Another recent study analyzed the anticholinesterase potential of an alkaloid extracted from fruits of *Melocanna baccifera* (Roxb.) Kurz, a native bamboo from north-east India, Bangladesh, Nepal, and Myanmar. The major constituent isolated from fruits was identified as Verbacine. This compound, in acetylcholinesterase (AChE) inhibition assay, showed IC_{50} of 16.01 μg/mL, very close to the observed for the standard Donepezil (12.91 μg/mL) (Govindan et al. 2018).

Moreira (2019) analyzed the anticholinesterase (AChE and BChE) potential of the extracts from leaves and culms of *Merostachys nessii*, a native Brazilian bamboo species. For AChE inhibition assay the lowest IC_{50} reported was for aqueous extract from leaves (446.79 μg/mL), followed by hydroethanol extract (624.22 μg/mL), and hexane extract (1036.16 μg/mL). *M. neesii* extracts were not able to inhibit the action of butyrylcholinesterase enzyme (BChE).

In the present study the lowest IC_{50} observed were for all analyzed extracts of *Merostachys pluriflora* (MP), all of them presented IC_{50} below 500 μg/mL (Table 2.2). The IC_{50} for MPE (401.11 μg/mL), MPA (423.24 μg/mL), and MPH (494.21 μg/mL) were very similar to the reported for *M. neesii* aqueous extract (446.79 μg/mL) (Anselmo-Moreira 2019).

In the aqueous and hydroethanol extracts of MP a diversity of secondary metabolites was identified. Most of the constituents identified in MP were also observed in the other studied species. However, some of them, as Feruloyltyramine, Chiococcasaponin IV, Vitexin -4"-O-glucoside, Rhoifolin, 3-*p*-coumaroylquinic acid, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine were detected only in MP (see *Chapter 1*, Table 1.3, page 74). Perhaps the presence of these constituents has contributed to the better performance of this species in this bioassay.

Flavonoids are emerging as promising candidates as AChE inhibitors (Khan et al. 2018). Some of them have shown a high capacity to inhibit the activity of the acetylcholinesterase. Besides that, they also have high antioxidant capacity and low toxicity, some advantages of this group of molecules for the use in animals (Uriarte-Pueyo and Calvo 2011).

Choi et al. (2014) evaluated the potential of Apigenin and its *C*-glycosylated derivatives on the inhibitory activity against cholinesterases (both AChE and BChE). All three compounds (Isovitexin, Vitexin, and Apigenin) showed promising anticholinesterase potential. However, the authors observed that the *C*-glycosylated derivatives had lower IC_{50} when compared with

Apigenin, and isovitexin was found as the most potent inhibitor of both AChE and BChE among these tested compounds.

In all bamboo species here studied, a diversity of *C*-glycosylated flavones was observed, being detected Isovitexine, Vitexin, Isoorientin, Violanthin, Vicenin, Swertisin, Isoscoparin, and others *C*-glycosylated flavones (see *Chapter 1*, Table 1.3, page 74).

Although these species presented a great diversity of secondary metabolites, some of these metabolites are presented in low amounts, which make the isolation of these substances unfeasible, nedding a large amount of plant matrix, as well as large quantities of solvents involved in extraction and purification, a procedure financially and ecologically expensive. However, these extracts would be interesting as potential herbal medicines.

The synergistic effects and polypharmacological action of a plant extract has increased its interest as potential therapeutic agents, and the use of a mixture of natural products to treat diseases has a number of interesting outcomes (Gibbons 2003).

Gibbons (2003) mentioned several extracts of plants that are used for the treatment of different diseases, and according this authour opinion there are an enormous potential to develop and use plant extracts as therapeutic agents, but it is important to develop a quality control area for plant extracts.

Although the IC_{50} found in the present study are considered high, it is worth remembering that this is the first study that reports the anticholinesterase activity of these Brazilian bamboo species.

2.3.2. *In vivo* biological assays

2.3.2.1. Zebrafish embryos survival rate and larvae morphological measures

Zebrafish embryos were exposed to aqueous extracts (0.1, 0.5, 1.0 and 1.5 mg/mL) immediately after fertilization for five days, and daily survival rate was recorded. The extracts of all tested bamboo species in their highest concentration (1.0 and 1.5 mg/mL) showed a toxicological effect in zebrafish larvae (Figure 2.4).

All animals treated with 1.5 mg/mL of the OGA and AAA extracts died with 1-day post fertilization (dpf), while the animals treated with the concentration of 1.0 mg/mL died in the second day. Reggarding the lower extract concentrations, 91% of the embryos treated with 0.1 mg/mL of OGA and 93% of AAA extracts, survived.

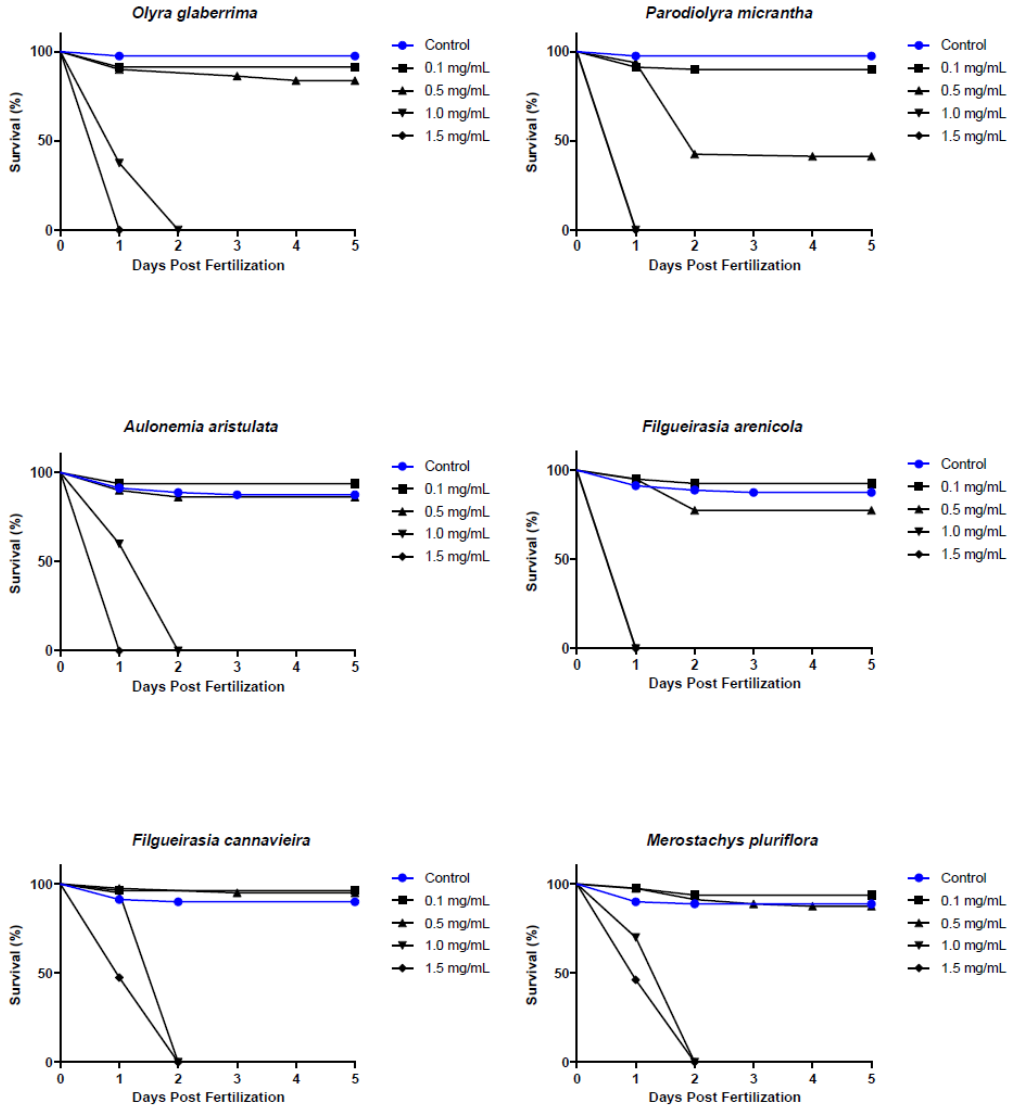

Figure 2.4 –Survival rate (%) of zebrafish embryos treated with different concentrations of Brazilian bamboo aqueous extracts. n = 2.400 embryos.

Zebrafish embryos treated with 1.5 mg/mL and 1.0 mg/mL of the PMA and FAA survived only on the first day of exposure. The survival rate for the animals treated with 0.5 mg/mL decreased 60% with 2 dpf for PMA and 23% for FAA. With 5 dpf 90% of the animals treated with 0.1 mg/mL of PMA extract survived. For FAA 92% of embryos exposed to a concentration of 0.1 mg/mL survived.

All embryos treated with 1.5 mg/mL and 1.0 mg/mL of FCA and MPA survived only until the second day of exposure. The survival rate of embryos treated with 0.1 mg/mL and 0.5 mg/mL was 96% and 95%, respectively for FCA. Regarding the lower concentration of MPA extract, 87% and 93% of the embryos survived when treated with 0.5 mg/mL and 0.1 mg/mL, respectively.

Potential toxicity of bamboo extracts was estimated by monitoring morphological alterations in 5 dpf larvae under a stereomicroscope. Some parameters like Body length (μm), ocular distance (μm), and surface area of the eyes (μm^2) were evaluated. For all extracts 30 animals was used to evaluated these parameters, with the exception of PMA at 0.5 mg/mL which, due to high mortality, only 15 larvae were used for these analyzes.

For body length, larvae exposed to OGA (p = 0.0055), AAA (p = 0.0485), and MPA (p = 0.0040) at 0.5 mg/mL showed a smaller body size when compared to larvae on the control group (Figure 2.5).

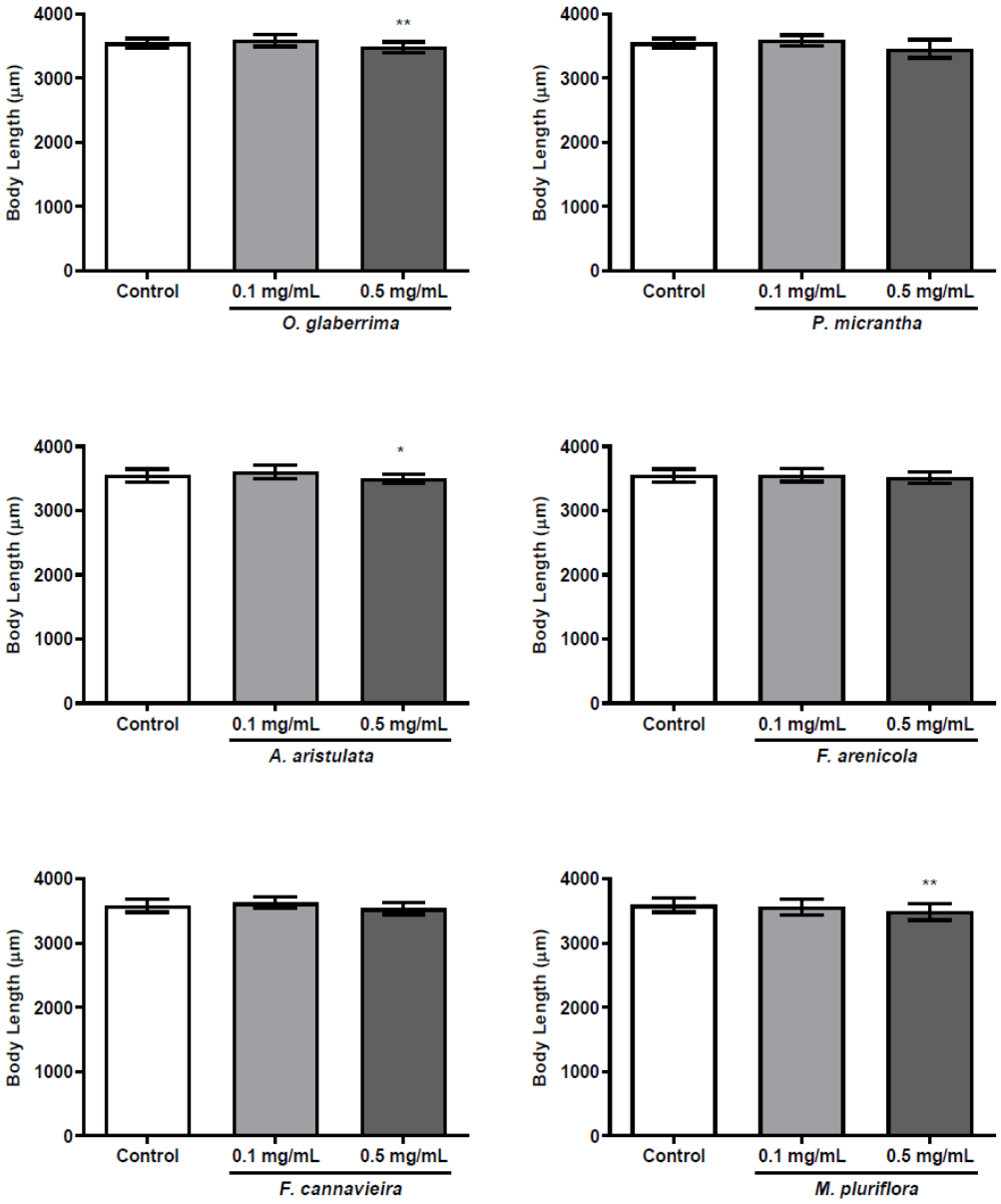

Figure 2.5 – Body length (μm) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL): n = 30; PM (0.1 mg/mL): n = 30; and (0.5 mg/mL): n = 15.

However, zebrafish larvae exposed to AAA (p = 0.0033), FA (p = 0.0017), and FCA (p = 0.0077) at 0.5 mg/mL increased their ocular distance when compared to the control group (Figure 2.6).

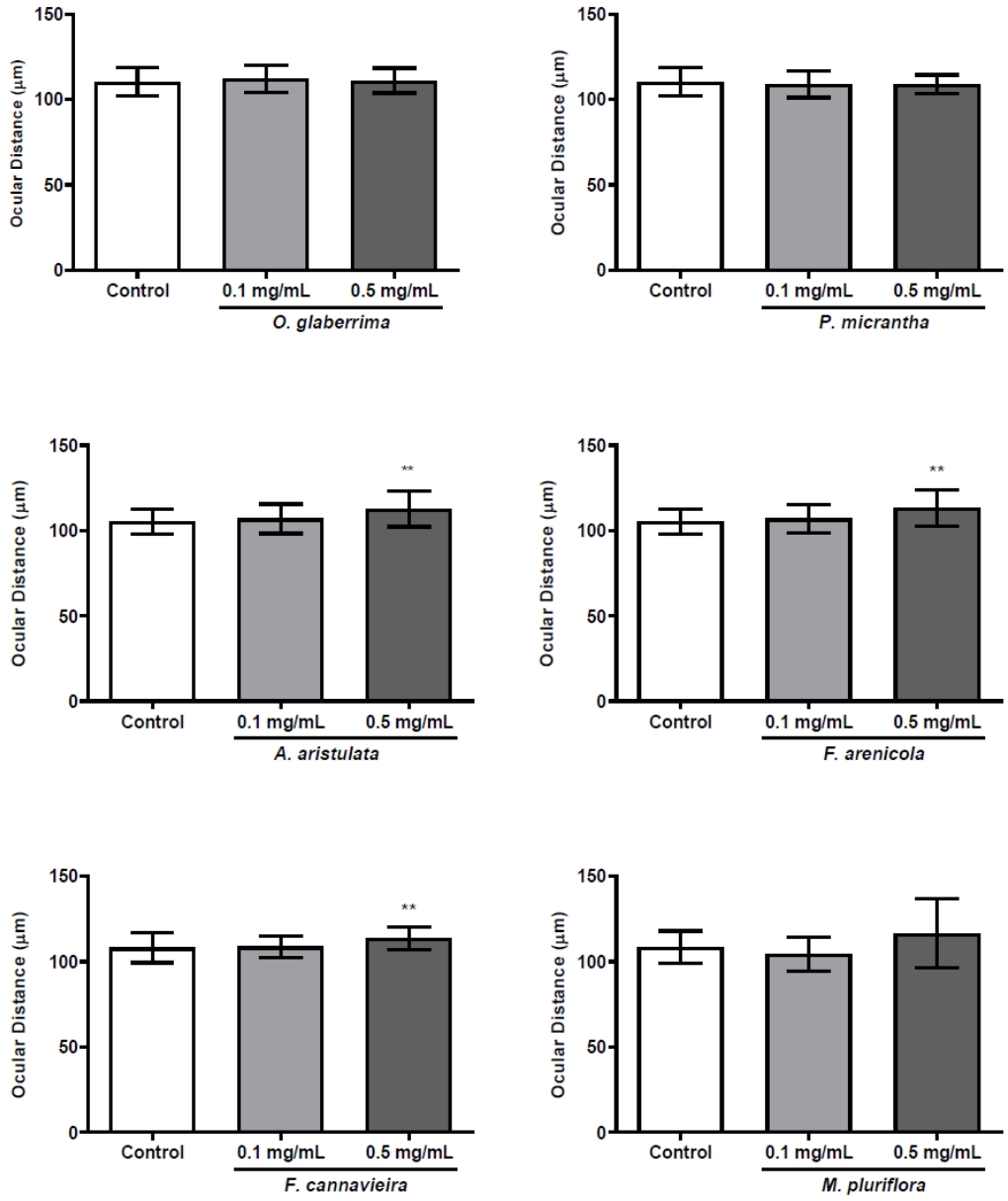

Figure 2.6 – Ocular distance (µm) of the zebrafish larvae treated with different concentrations od Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$.
OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL): n = 30 / PM (0.1 mg/mL): n = 30; and (0.5 mg/mL): n = 15.

Larvae exposed to 0.5 mg/mL of the OGA ($p < 0.0001$), PMA ($p = 0.0385$), AAA ($p < 0.0001$), FAA ($p < 0.0001$), FCA ($p = 0.0109$), and MPA ($p < 0.0001$) extracts showed significant smaller body surface area when compared to the control group. As well as larvae exposed to 0.1 mg/mL of FAA ($p = 0.0194$) extract presented the same result (Figure 2.7).

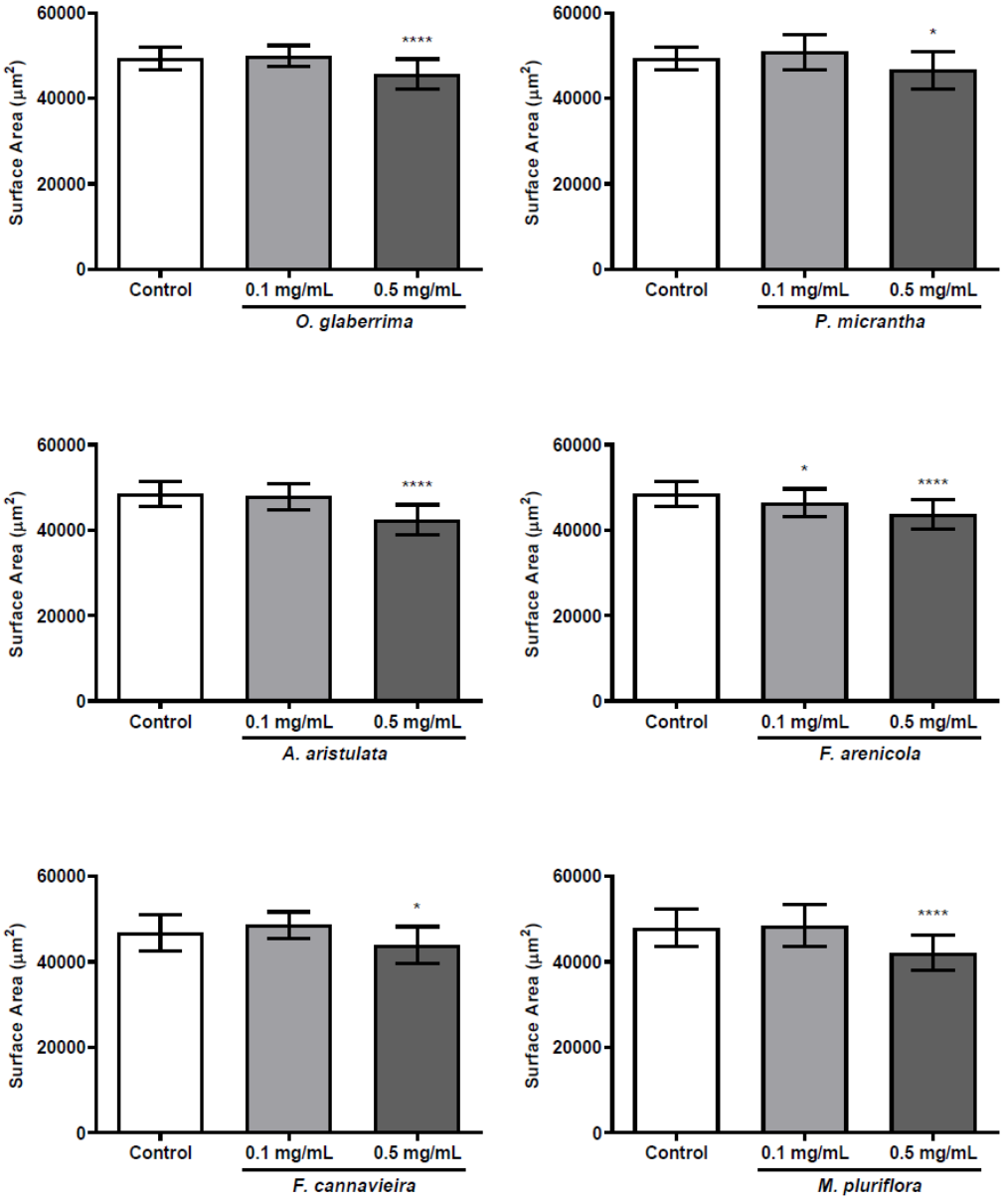

Figure 2.7 – Surface area (µm²) of zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$.
OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL): n = 30 / PM (0.1 mg/mL): n = 30; and (0.5 mg/mL): n = 15.

For a drug or a plant extract to be considered safe for medicinal use, it is necessary to assess its toxicity. Zebrafish offers several practical advantages as a model organism for toxicological research. They are small, less expensive to maintain in control experiments, and easily bred in large numbers. Drug administration is simple, zebrafish larvae can absorb small molecules diluted in water through their skin and gills. As well as drugs can be delivered orally for assays performed after larvae stage. Besides that, female zebrafish can produce hundreds of eggs weekly, so large sample sizes are easily achieved, allowing for statistically powerful dose-response studies (McGrath and Li 2008; Truong et al. 2011).

It was possible to infer that the concentrations of 0.1 mg/mL and 0.5 mg/mL were the safest concentrations for a subchronic exposure to the aqueous extracts of the studied bamboo species.

Through morphological analysis it was possible to conclude that the 0.5 mg/mL of the bamboo aqueous extracts were not lethal to the animals, but it was able to cause significant morphological changes in the larvae. Perhaps, for a subchronic exposure, this concentration is still too high for the zebrafish larvae.

2.3.2.2. Larvae exploratory and avoidance behavior analysis

The animals were individually submitted for a session of exploratory behavior analysis. For almost all extracts in both concentrations (0.1 and 0.5 mg/mL), about 30 to 36 larvae were used, with the exception of PMA at 0.5 mg/mL, 18 larvae were used for these analyzes.

For almost all studied species the extracts at 0.5 mg/mL induced a significant decrease in total distance traveled, with the exception for PMA, as seen in Figure 2.8.

Zebrafish larvae treated with OGA, AAA, FCA, and MPA at 0.5 mg/mL ($p < 0.0001$), as well as FAA ($p = 0.0190$), traveled shorter distances when compared to the control group (Figure 2.8).

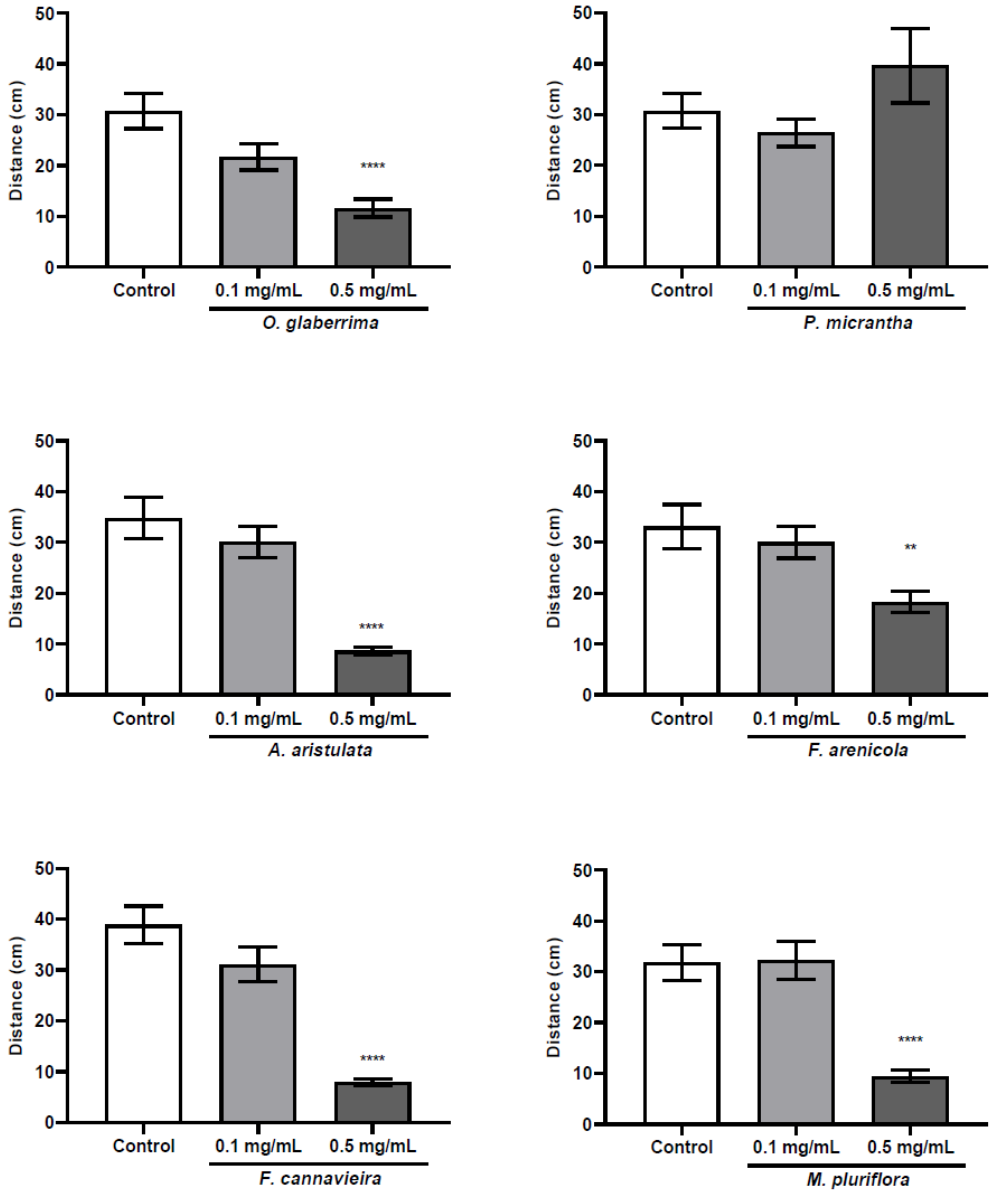

Figure 2.8 – Distance traveled (cm) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL) and PM (0.1 mg/mL): n = 30-36; PM (0.5 mg/mL): n = 18.

However, only the larvae treated with 0.5 mg/mL of the AAA (p = 0.0011) and OGA (p = 0.0328) extracts showed an increase in swimming speed than the control group (Figure 2.9).

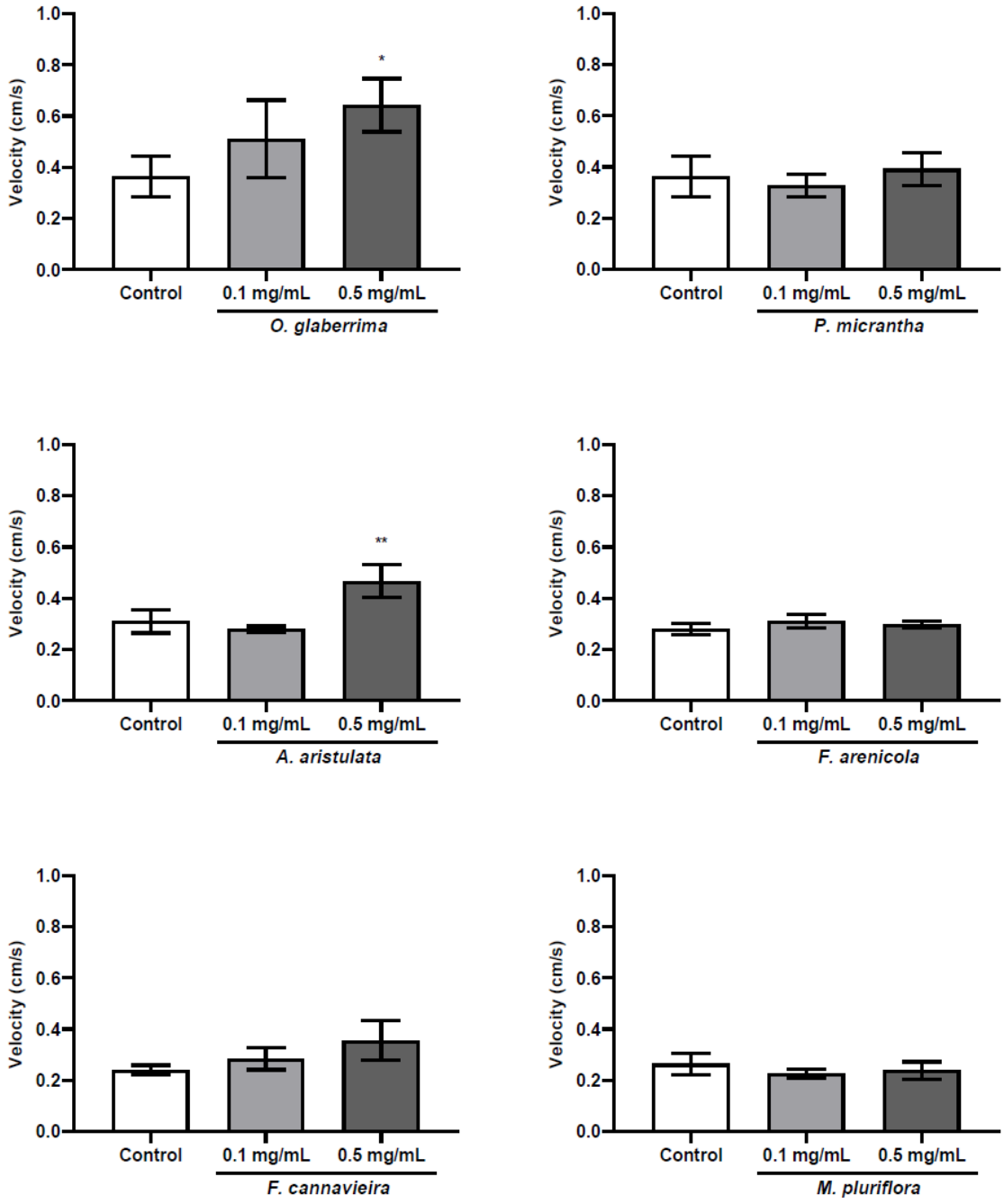

Figure 2.9 – Velocity (cm/s) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL) and PM (0.1 mg/mL): n = 30-36; PM (0.5 mg/mL): n = 18.

About the time that the animals remained in movement exploring the environment during the assay, it was possible to observe that animals treated with OGA, AAA, FCA, MPA (p < 0.0001), and FAA (p = 0.0020) at 0.5 mg/mL; and FCA at 0.1 mg/mL (p = 0.0432) moved much less when compared to the animals in the control group (Figure 2.10).

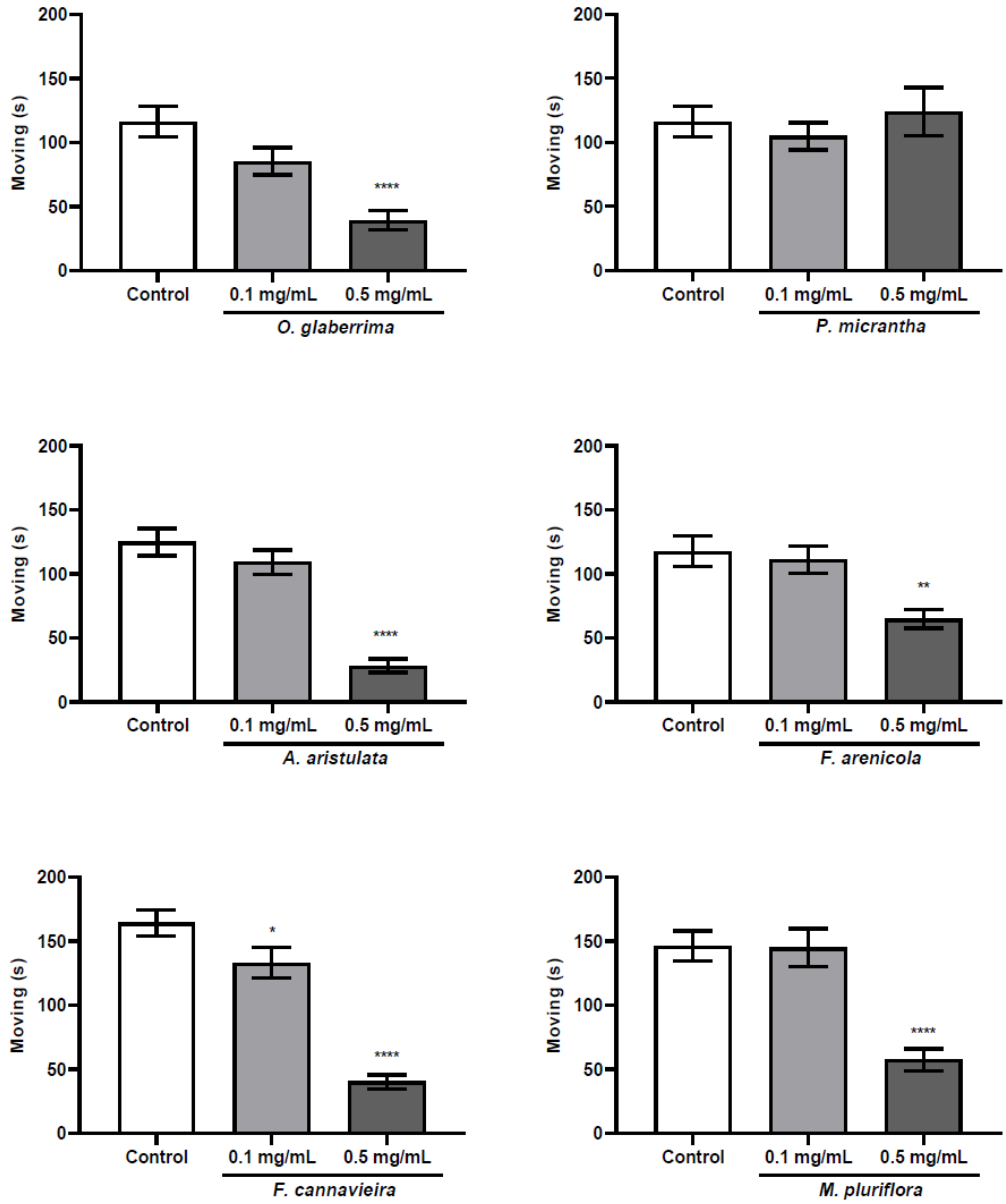

Figure 2.10 – Time moving (s) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL) and PM (0.1 mg/mL): n = 30-36; PM (0.5 mg/mL): n = 18.

The time spent in each plate-well position (outside *vs*. inside area) was considered an index of anxiety. However, for this parameter, the zebrafish larvae treated with AAA (p = 0.0464) and OGA (p = 0.0470) at 0.5 mg/mL showed significant differences in their behavior when compared to the control group (Figure 2.11).

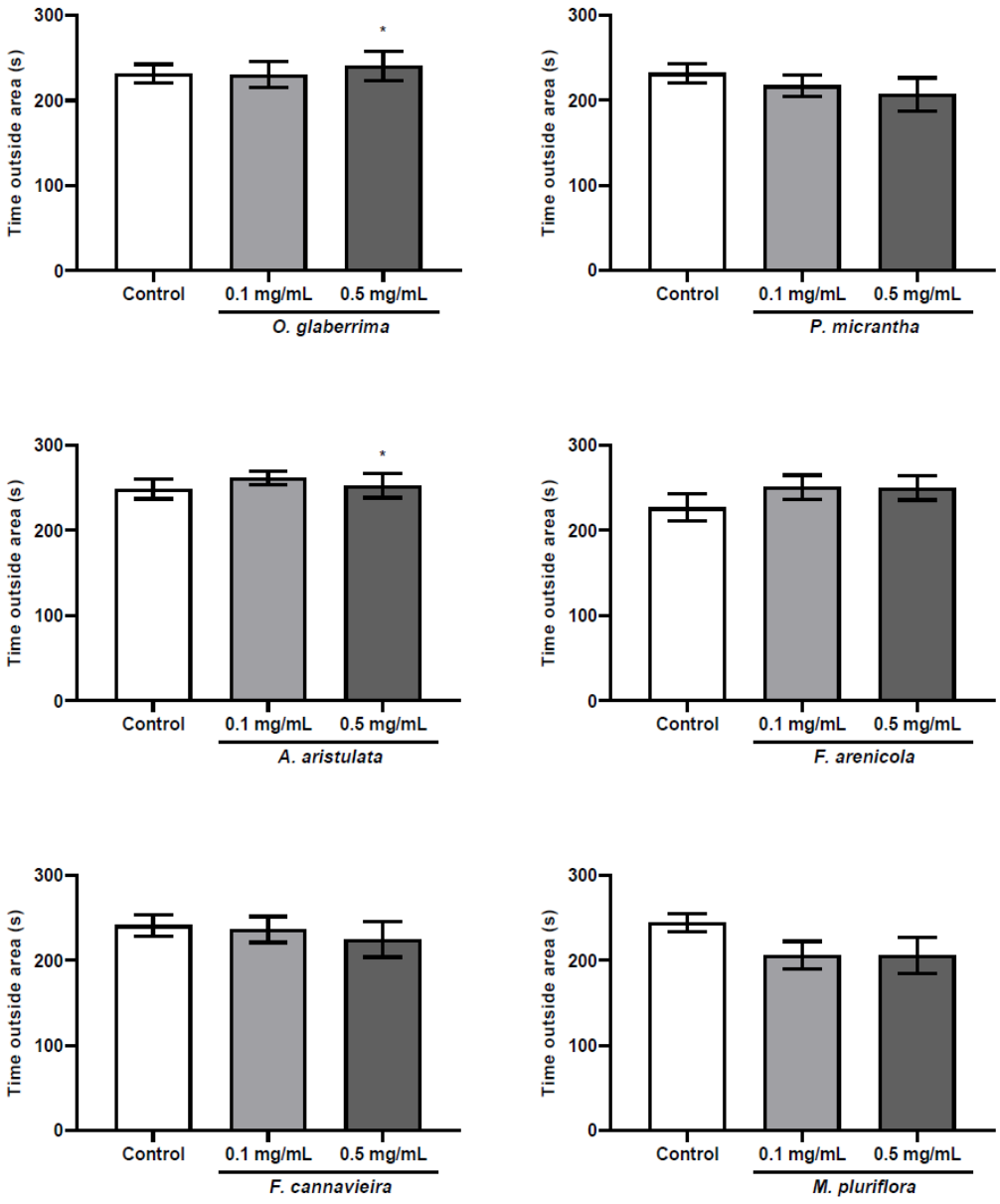

Figure 2.11 – Time outside area (s) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL) and PM (0.1 mg/mL): n = 30-36; PM (0.5 mg/mL): n = 18.

The larvae treated with 0.5 mg/mL of OGA, AAA, FCA ($p < 0.0001$), FAA ($p = 0.0068$), and MPA ($p = 0.0251$) extracts presented higher absolute turn angle than the control group (Figure 2.12).

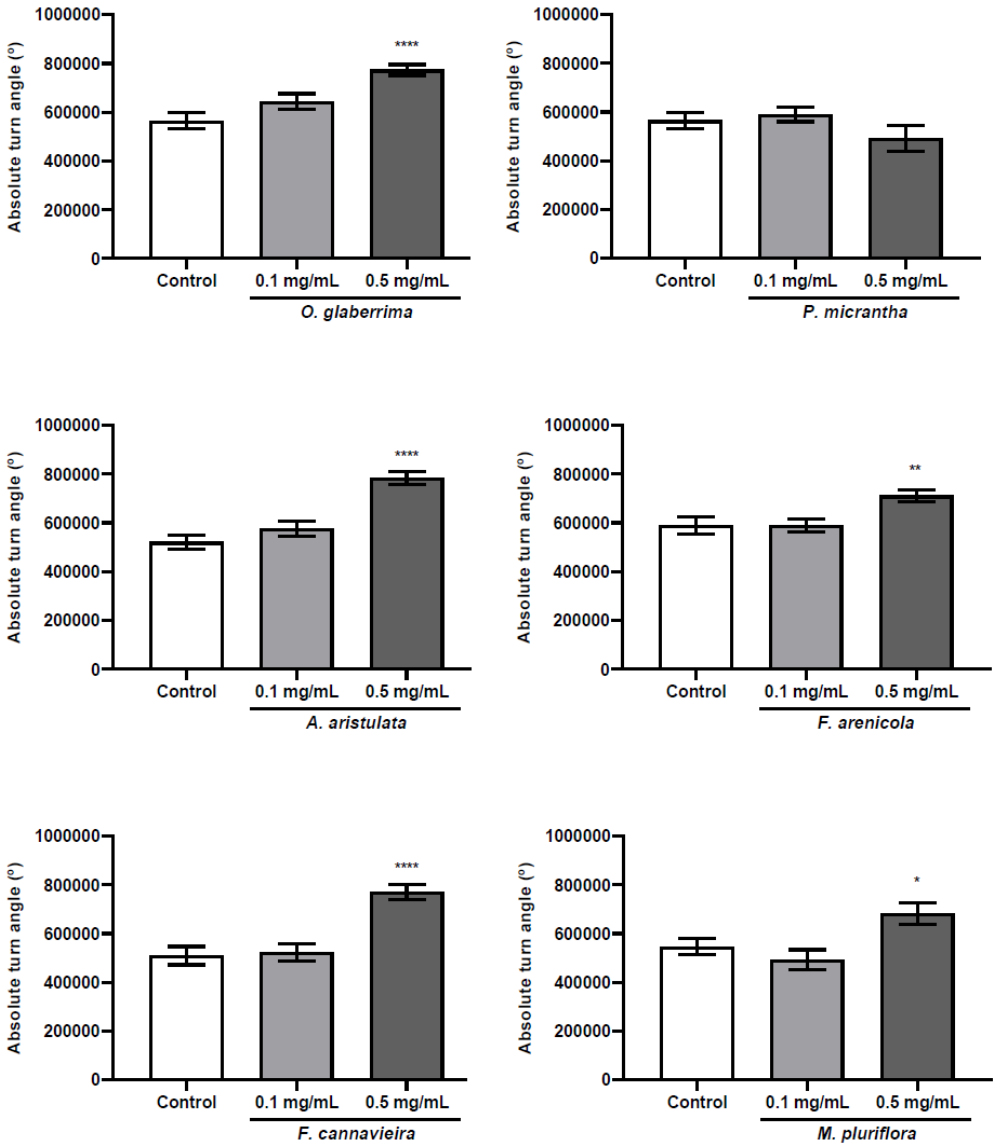

Figure 2.12 – Absolute turn angle (º) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, AA, FA, FC, MP (0.1 and 0.5 mg/mL) and PM (0.1 mg/mL): n = 30-36; PM (0.5 mg/mL): n = 18.

High conservation between zebrafish and human brain organization, besides a great degree of similarity between their neuroanatomic and neurochemical pathways is emerging zebrafish as an increasingly successful model for translational research on human neurological disorders (Saleem and Kannan 2018).

The simplicity of the zebrafish larvae motor organization provided a complete ethogram descrition of the larvae, resulting in an exhaustive list of its behavior patterns. The sophisticated tracking and behavioral analysis tools, can accurately record each movement of the analyzed larvae (Orger and Polavieja 2017).

Immediately after hatching, the zebrafish larvae exhibit vigorous locomotor activity and predatory behavior. This simple predatory behavior involves several neural processes, including visual perception, recognition, decision making, and motor control (Siebel et al 2015). Therefore, zebrafish locomotor activity can be analyzed for assessing drug-induced neurotoxicity, and the alterations in the locomotion behavior may indicate changes in neuronal functions (McGrath and Li 2008).

Some authors have evaluated the neurotoxic effect of different compounds, including fungicides (Altenhofen et al. 2017), pesticides (Altenhofen et al. 2019), and heavy metals (Nabinger et al. 2018), on the exploratory behavior of zebrafish larvae exposed to such agents. All of them observed a decrease in exploratory behavior caused by these compounds.

When exposed to the highest concentration of the tested fungicide the animals presented reductions in distance traveled, absolute turn angle, and time outside the area of the well (Altenhofen et al. 2017). In the study that evaluated the effect of the pesticide, the zebrafish larvae showed a reduction in distance traveled, mean speed, and time mobile (Altenhofen et al. 2019).

In the present study, it was also observed a decrease in the exploratory behavior of zebrafish larvae exposed to 0.5 mg/mL of the bamboo extracts. The animals traveled shorter distances and had less time in movement; besides they presented a greater turning angle when compared with the animals in the control group. Therefore, according to the analyzed parameters, we can infer that the bamboo extracts in this concentration caused a neurotoxic effect in the animals.

The location of the larvae in the wells of the plates is used as a parameter for assessing anxiety, as the zebrafish tends to prefer the corners of the plate (Siebel et al. 2015). However, for this parameter, no significant differences were observed between treated and control groups, so the extracts in the tested concentrations did not change animals' anxiety behavior.

After exploratory analyses, larvae were submitted to avoidance-escape behavior from a visual stimulus. The number of larvae on the non-stimuli area during the 5 minutes session was considered an indicative of their cognitive ability.

There were significant changes in the larvae avoidance response after bamboo extracts treatment (Figure 2.13). Treatements with both 0.1 mg/mL and 0.5 mg/mL concentrations of the

OGA ($p < 0.0001$; $p = 0.0063$), PMA ($p = 0.0014$; $p = 0.0141$), and FAA ($p = 0.0008$; $p = 0.0131$) extracts showed higher percentage of animals in the non-stimuli area when compared to the control group. However, only the animals treated with 0.5 mg/mL of the AAA ($p = 0.0038$) extract showed significant differences for this parameter (Figure 2.13).

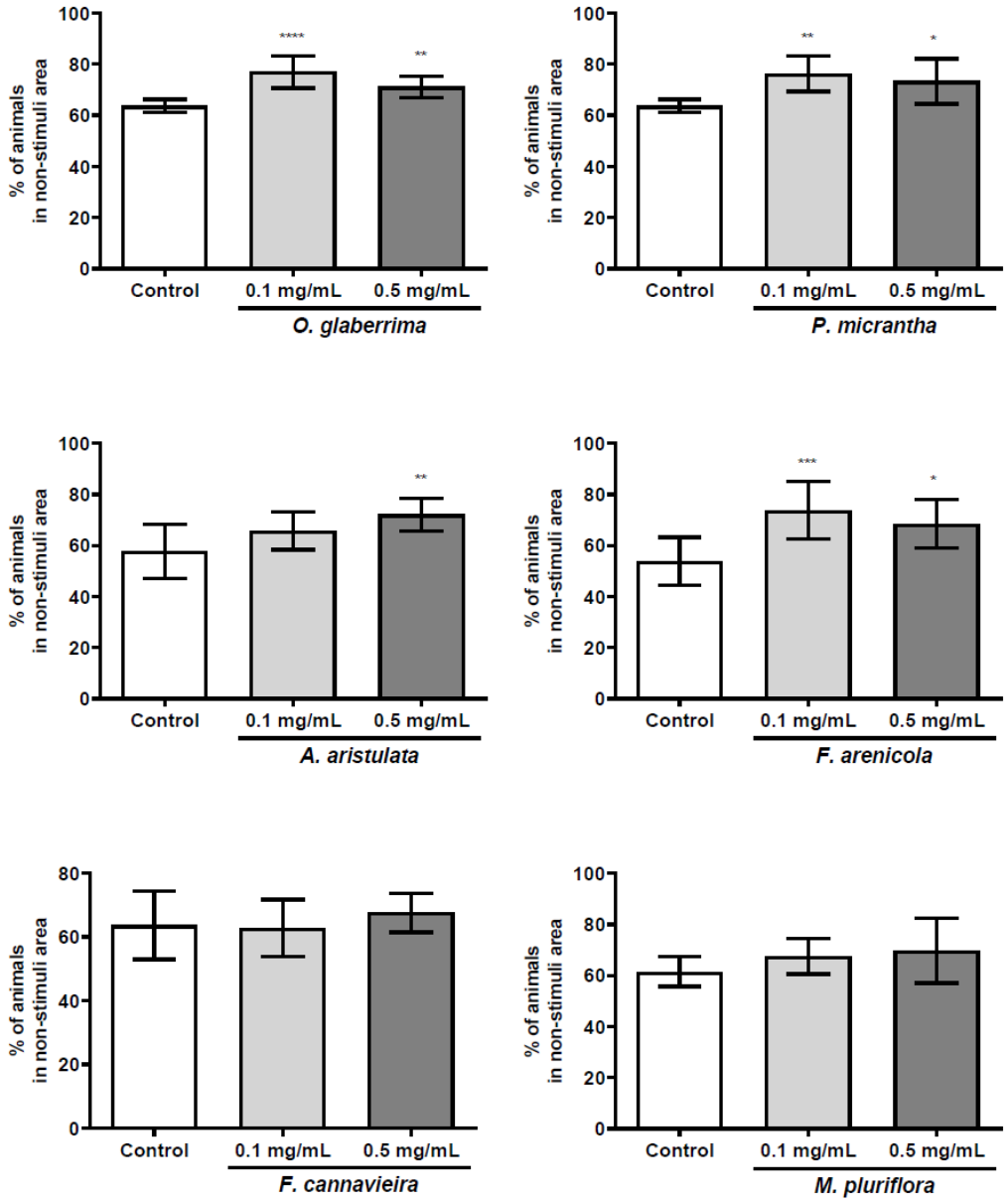

Figure 2.13 – Percentage of the zebrafish larvae in non-stimuli area during avoidance behavior analysis after exposure to different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, PM, AA, FA, FC, MP (0.1 and 0.5 mg/mL): n = 18.

It is known that zebrafish larvae have an accurate vision, so studies have used projections on the computer screen to analyze spontaneous behavior in response to the projected aversive stimulus (Siebel et al. 2015). Escape behavior is innate against an aversive stimulus, these reflexive responses have very short latencies, but have surprising layers of complexity.

It was possible to note that in both concentrations tested (0.1 mg/mL and 0.5 mg/mL), the extracts of OGA, PMA, and FAA increased the percentage of animals with escaping responses from an aversive stimulus, as also 0.5 mg/mL of AAA extract, when compared to the control group (Figure 2.13).

Animals with their cognitive ability affected showed a decrease in the percentage of escaping responses from an aversive stimulus (Nabinger et al. 2018; Altenhofen et al. 2019). However, in the present study an increase in escaping responses was observed, so it was possible to infer that OGA, PMA, FAA, and AAA extracts increased the cognitive capacity of the tested zebrafish larvae.

2.3.2.3. Inhibitory avoidance task

Next step was to evaluate the performance of adult zebrafish in the inhibitory avoidance task. This assay evaluates the potential protective effect of an extract on scopolamine-induced memory impairment. Saline-exposed animals followed by water treatment demonstrated robust retention of memory during the test session performed 24 h after training ($p < 0.05$).

Pretreatment of OG, PM, AA, FC, and MP, at 0.1 and 0.5 mg/kg, followed by water treatment, resulted in significant differences between zebrafish training and test sessions, thus suggesting effective learning of the task. The results demonstrated that animals pretreated with FA at 0.1 and 0.5 mg/Kg followed by water treatment presented a cognitive impairment since there were no differences between training and test sessions. The other extracts did not alter the memory performance (Figure 2.14).

Saline-exposed animals subsequently treated with scopolamine did not exhibit memory retention during the test session performed 24 h after training. Interestingly, treatment with FC, MP (0.1 and 0.5 mg/Kg), and OG (0.1 mg/kg) prevented the memory impairment induced by pre-training scopolamine exposure, as observed by the difference in latencies between training and test sessions for each treatment ($p < 0.05$), suggesting that these extracts are able to reduce the memory deficits caused by scopolamine (Figure 2.14).

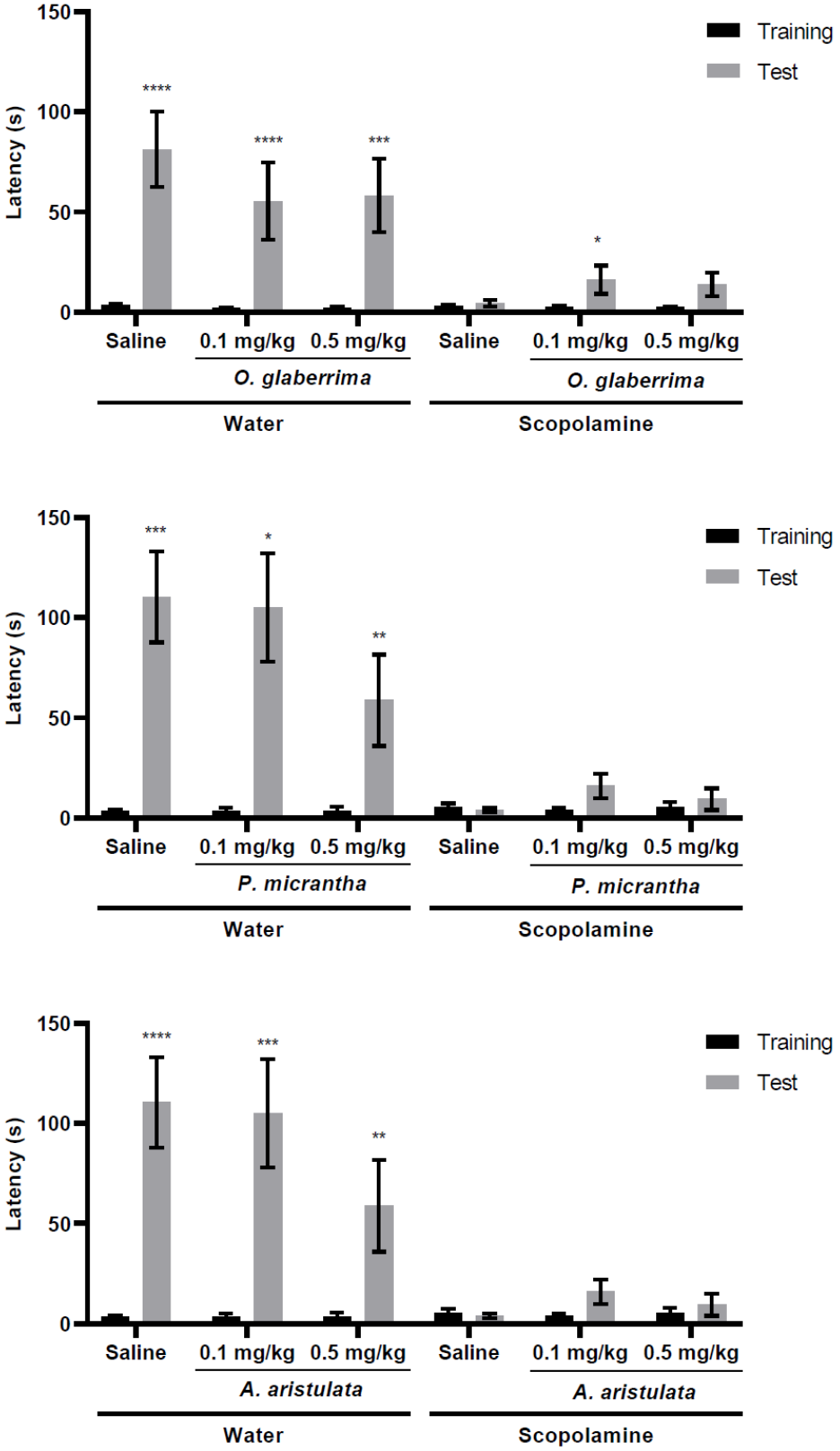

Figure 2.14 – Latency (s) between training and test session of the animals on inhibitory avoidance analysis after exposure to different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, PM, AA, FA, FC, MP (0.1 and 0.5 mg/Kg): n = 10.

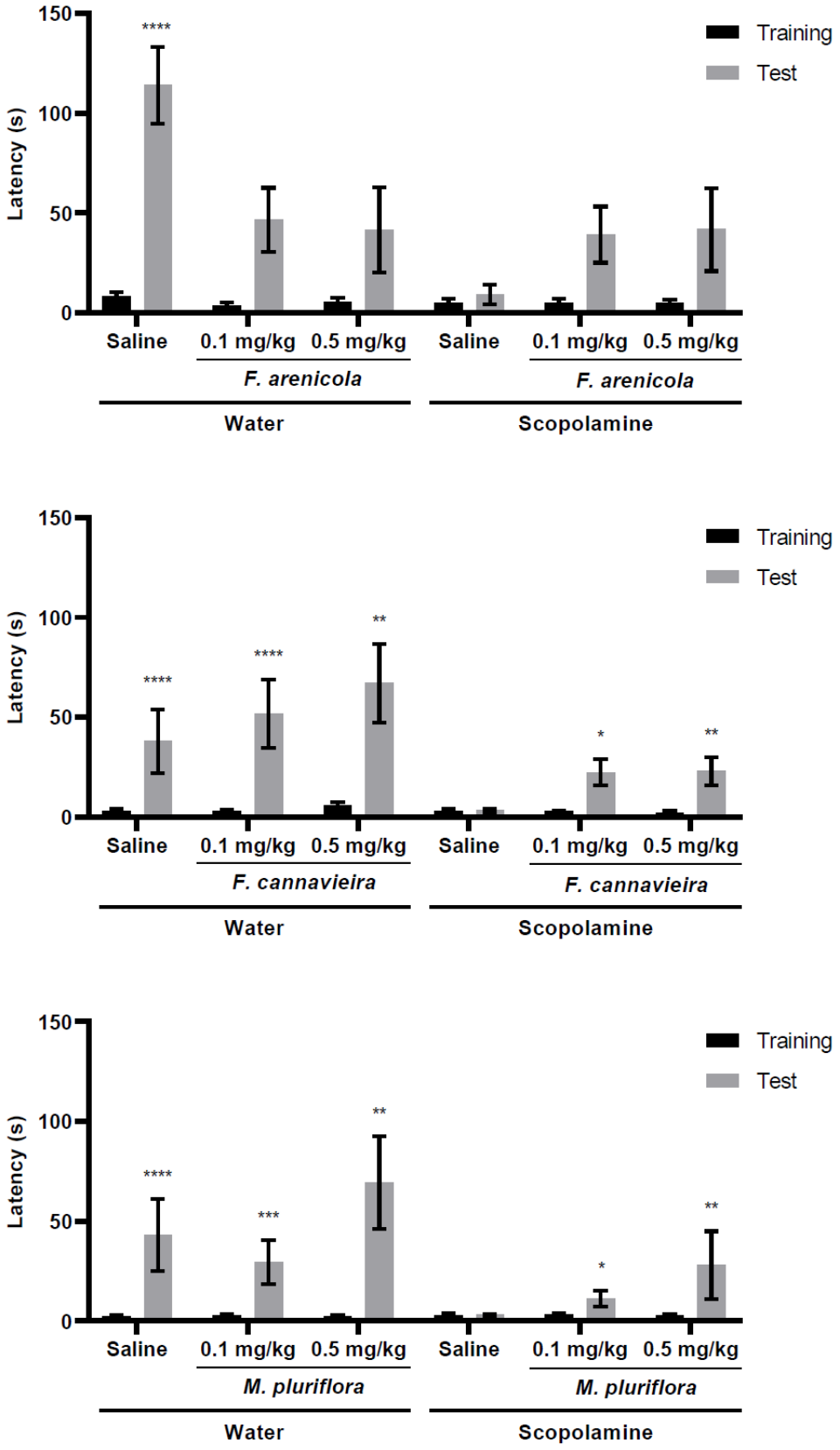

Figure 2.14 – Continuation.

The adult zebrafish can distinguish light and dark compartments. And it is known that the zebrafish has a preference for dark environments, in which the animal spends a longer period during its natural behavior (Siebel et al. 2015).

The zebrafish inhibitory avoidance protocol proved to be efficient for studies of memory in adult zebrafish. The memory acquired by the zebrafish after the testing session is evident and permanent, so this protocol is considered efficient in pharmacological and toxicological studies that aimed to analyze mechanisms involved in neuronal diseases (Siebel et al. 2015).

Zebrafish proved to be an excellent model for studies of neurological diseases. Natural products have been a prolific source of molecules for the treatment of different diseases. In this sense, different studies have analyzed the potential of natural products using zebrafish as an experimental model.

Richetti et al. (2011) tested the neuroprotective effect of two well-known flavonoids, quercetin and rutin, using the zebrafish inhibitory avoidance protocol. The authors observed that zebrafish adults pre-treated with quercetin or rutin 1 h before the beginning of scopolamine treatment had less memory impairment caused by scopolamine.

In another study, the authors evaluated the combined effects of *Cynodon dactylon* (L.) Pers. and *Brassica juncea* (L.) Czern. extracts on cognition using scopolamine-induced amnesia in the zebrafish model. They observed that there were no differences in the memory retention of the animals treated with each of the extracts individually, however, when combined the extracts prevented scopolamine-induced amnesia (Yendapalli et al. 2019).

It is worth mentioning that this is the first study that evaluated the toxicity and biological potential of the Brazilian bamboo extracts using zebrafish as a model.

2.4. Conclusions

In conclusion, hydroethanol and aqueous extracts of the six Brazilian bamboo species here studied have a high *in vitro* antioxidant potential, especially *P. micrantha* (PMA, PME) and *F. arenicola* (FAA, FAE) with the lowest EC_{50}. *M. pluriflora* (MPA, MPE) showed a moderate potential as acetylcholinesterase inhibitors.

The *in vivo* assays showed that aqueous extracts at 0.1 mg/mL are not toxic for the zebrafish embryos, as well as they did not alter the exploratory behavior of the zebrafish larvae. Besides that, OGA, PMA, AAA, and FAA extracts increased the cognitive capacity of zebrafish

larvae in the face of an aversive stimulus. Furthermore, FCA and MPA and OGA were able to prevent the memory loss of the adult zebrafish after exposed to scopolamine.

However, even though they are promising species, studies on the cultivation and reproduction of Brazilian bamboo species are still insufficient, so studies are necessary to assess whether these species are possible to be cultivated, or whether their sustainable management would be viable.

Therefore, the Brazilian bamboo species, as the Asian ones, showed potential for further investigating regarding the possible benefits of using these extracts to improve cognitive and memory capacity, which are commonly affected by different neurological diseases.

Final Considerations

1. Principal results

The figure below summarizes the principal results obtained in this study:

Olyreae (herbaceous bamboo)

Both herbaceous species showed:

- ❖ high levels of flavones and soluble sugars
- ❖ the highest yield of extracts
- ❖ aqueous extracts increased the cognitive capacity of zebrafish larvae

Olyra glaberrima

- higher levels of fatty acids, cardenolides, isoflavones, diterpenoids, and glycerides
- aqueous extract at 0.5 mg/mL caused changes in the morphology and also in the exploratory behavior of the zebrafish larvae
- aqueous extract prevented the memory deficit induced by scopolamine treatment

Parodiolyra micrantha

- higher levels of total phenols and flavonoids
- higher levels of aurones
- the hydroethanol extract showed the lowest EC_{50} in ORAC assay

Bambuseae (tropical woody bamboo)

❖All woody species showed high levels of flavones and flavonols

Aulonemia aristulata

- higher levels of amino acids and derivatives, sphingolipids, and cardenolides
- aqueous extract at 0.5 mg/mL caused changes in the morphology and also in the exploratory behavior of the zebrafish larvae
- aqueous extract increased the cognitive capacity of zebrafish larvae

Filgueirasia arenicola

- hydroethanol extract showed the lowest EC_{50} in DPPH assay
- aqueous extract at 0.5 mg/mL caused changes in the morphology and also in the exploratory behavior of the zebrafish larvae
- aqueous extract increased the cognitive capacity of zebrafish larvae

Filgueirasia cannavieira

- higher levels of lignans
- aqueous extract at 0.5 mg/mL caused changes in the morphology and also in the exploratory behavior of the zebrafish larvae
- aqueous extract prevented the memory deficit induced by scopolamine treatment

Merostachys pluriflora

- all extracts showed the lowest IC_{50} in AChE assay
- aqueous extract at 0.5 mg/mL caused changes in the morphology and also in the exploratory behavior of the zebrafish larvae
- aqueous extract prevented the memory deficit induced by scopolamine treatment

2. General conclusions

This was the first study of chemical characterization with these six bamboo species and the first study with herbaceous bamboo species. Furthermore, this was the first study that used zebrafish as experimental model to test bamboo extracts.

This study allowed the screening of the best concentrations for *in vivo* tests, since all of bamboo extracts showed to be toxic to zebrafish embryo at 1.0 and 1.5 mg/mL. Besides that, it was noted that the aqueous extracts at 0.5 mg/mL from almost all studied species are high for subchronic exposure, causing morphological and behavioral changes in zebrafish larvae at this concentration.

All species in this study showed potential as a natural source of antioxidant molecules. Four of the six studied species showed potential to increase cognitive ability in zebrafish larvae.Whitin the six Brazilian bamboo species, *M. pluriflora* proved to be a very promising species for future studies to treat memory, due to the good performance in acetylcholinesterase and inhibitory avoidance task here performed.

However, further studies are needed to understand which molecules are responsible for these activities, as well as their mechanism of action and bioavailability.

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Supplementary data: Chapter 1

Table S.1.1 – Standard curves used for the quantification of total phenols and flavonoids in colorimetric methods.

Assay	Reference compound	Amount	Line equation	R^2
Total phenol content	Gallic acid	0.25 µg - 4 µg	y = 0,1515x + 0,0664	0.9944
Total flavonoid content	Quercetin	0.36 µg - 8.4 µg	y = 0,2522x + 0,0441	0.9994

Table S.1.2 – Contents of constituents detected by GC-EIMS in the polar extracts of leaves from six Brazilian bamboos expressed as milligram of ribitol equivalent per g of plant material (mg/g RE). **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata;* **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora.*

Peak	R.T	Constituent	mg/g Ribitol equivalent (RE)					
			OG	PM	AA	FA	FC	MP
1	14.91	Norvaline (2TMS)	0.09	0.11	0.14	0.00	0.00	0.03
2	14.97	Valine (2TMS)	0.03	0.00	0.00	0.00	0.00	0.00
3	15.84	Urea (2TMS)	0.01	0.01	0.02	0.01	0.01	0.00
4	16.17	Serine (2TMS)	0.54	0.25	0.55	0.02	0.00	0.02
5	16.30	Ethanolamine (3TMS)	0.32	0.15	0.19	0.15	0.18	0.17
6	16.58	Glycerol (3TMS)	0.77	0.89	0.72	0.33	0.46	0.16
7	17.09	Isoleucine (2TMS)	0.03	0.05	0.05	0.00	0.00	0.01
8	17.17	Threonine (2TMS)	0.23	0.19	0.33	0.01	0.00	0.02
9	17.23	Proline (2TMS)	0.02	0.00	0.00	0.00	0.00	0.00
10	17.46	Maleic acid (2TMS)	0.07	0.03	0.00	0.01	0.02	0.01
11	17.75	Succinic acid (TMS)	0.54	0.07	0.11	0.05	0.09	0.03
12	18.11	Glyceric acid (3TMS)	0.21	0.16	0.16	0.03	0.10	0.14
13	18.50	Itaconic acid (2TMS)	0.23	0.12	0.06	0.02	0.02	0.05
14	18.73	Fumaric acid (2TMS)	0.07	0.06	0.05	0.02	0.25	0.03
15	20.03	Mesaconic acid (2TMS)	0.04	1.14	0.02	0.01	0.01	0.02
16	20.63	Aspartic acid (2TMS)	0.26	0.04	0.10	0.03	0.01	0.03
17	22.20	Malic acid (3TMS)	3.36	0.50	0.98	0.24	0.49	0.13
18	22.62	Erythritol (4TMS)	0.08	0.10	0.07	0.14	0.79	0.15
19	22.71	Salicylic acid (2TMS)	0.14	0.07	0.09	0.02	0.00	0.03
20	23.03	5-oxoproline (2TMS)	0.26	0.22	0.14	0.09	0.02	0.09
21	23.17	GABA (3TMS)	0.20	0.19	0.31	0.08	0.05	0.10
22	23.47	Threonic acid (4TMS)	0.13	0.27	0.21	0.03	0.03	0.12
23	23.70	Phenylalanine (TMS)	0.10	0.07	0.20	0.04	0.00	0.02
24	24.89	Asparagine (2TMS)	0.50	0.05	0.22	0.02	0.03	0.02
25	26.15	3.4.5-Trihydroxypentanoic acid (4TMS)	0.03	0.01	0.01	0.00	0.03	0.01
26	27.89	NI-1	0.00	0.00	0.00	0.00	0.00	0.00
27	28.22	Aconitic acid (3TMS)	0.12	0.33	0.04	0.01	0.00	0.18
28	28.40	Ribonic acid (5TMS)	0.06	0.05	0.04	0.02	0.02	0.03
29	28.77	NI-2	0.00	0.00	0.00	0.00	0.00	0.00

Peak	R.T	Constituent	mg/g Ribitol equivalent (RE)					
			OG	**PM**	**AA**	**FA**	**FC**	**MP**
30	29.25	Azelaic acid (2TMS)	0.05	0.04	0.04	0.03	0.03	0.04
31	29.62	Citric acid (4TMS)	0.44	0.24	0.19	0.05	0.06	0.15
32	30.35	NI-3	0.00	0.00	0.00	0.00	0.00	0.00
33	30.63	Fructose, *O*-methyloxime (5TMS)	8.97	12.71	1.89	0.28	0.51	1.05
34	31.13	Glucose, *O*-methyloxime (5TMS)	6.62	8.95	1.16	0.32	0.50	0.63
35	31.43	Galactose, *O*-methyloxime (5TMS)	1.12	1.82	0.21	0.06	0.09	0.12
36	31.75	Sorbitol (TMS)	0.18	0.51	0.09	0.24	0.19	0.11
37	32.18	*p*-Coumaric acid (TMS)	0.03	0.08	0.06	0.09	0.07	0.07
38	32.40	Gallic acid (4TMS)	0.22	0.13	0.11	0.08	0.11	0.12
39	33.13	Gluconic acid (6TMS)	0.05	0.06	0.01	0.04	0.02	0.02
40	33.33	(3-Hydroxy-4-methoxyphenyl)ethylene glycol (3TMS)	0.06	0.00	0.05	0.02	0.08	0.04
41	34.83	*Myo*-Inositol (6TMS)	0.10	0.09	0.06	0.01	0.01	0.03
42	38.75	Methyl galactoside (4TMS)	0.02	0.05	0.04	0.00	0.00	0.01
43	43.76	Sucrose (8TMS)	6.17	0.20	0.05	0.00	0.04	0.01
		Amino acid and derivatives:	2.25	1.17	2.04	0.30	0.11	0.34
		Soluble sugars:	24.03	25.32	4.30	1.38	2.59	2.28
		Organic acids:	5.45	3.08	1.96	0.59	1.25	1.00
		Organic compounds:	0.33	0.16	0.21	0.16	0.20	0.17
		Phenolic acids:	0.38	0.29	0.26	0.18	0.17	0.21

Table S.1.3 – Mass fragments and their relative abundances for constituents detected by GC-EIMS in polar and nonpolar extracts of leaves from six Brazilian bamboos.

Peak	R.T. (min)	Constituent	Experimental main fragments: *m/z* (%)
1	14.91	Norvaline TMS derivative	144 (999); 73 (724); 75 (178); 145 (131); 218 (129); 147 (117); 59 (93); 74 (75); 100 (62); 58 (61)
2	14.97	Valine TMS derivative	144 (999); 73 (805); 75 (192); 147 (158); 218 (139); 145 (132); 59 (103); 79 (98); 58 (93); 74 (93)
3	15.84	Urea TMS derivative	147 (999); 73 (616); 189 (454); 75 (370); 171 (270); 79 (251); 66 (185); 74 (183); 99 (177); 52 (160)
4	16.17	Serine TMS derivative	73 (999); 116 (671); 132 (644); 75 (322); 57 (194); 74 (131); 103 (128); 147 (122); 133 (101); 144 (92)
5	16.30	Ethanolamine TMS derivative	174 (999); 73 (671); 175 (185); 147 (167); 100 (155); 59 (138); 86 (135); 133 (81); 176 (79); 74 (60)
6	16.58	Glycerol TMS derivative	73 (999); 147 (413); 205 (261); 117 (207); 103 (204); 133 (152); 75 (101); 218 (97); 59 (93); 74 (87)
7	17.09	Isoleucine TMS derivative	158 (999); 73 (868); 75 (329); 147 (178); 218 (174); 100 (173); 79 (153); 159 (150); 52 (136); 59 (117)
8	17.17	Threonine TMS derivative	73 (999); 117 (408); 75 (263); 130 (227); 57 (215); 147 (165); 142 (152); 219 (124); 74 (110); 146 (110)
9	17.23	Proline TMS derivative	142 (999); 73 (739); 75 (247); 147 (164); 143 (143); 74 (99); 59 (88); 100 (86); 52 (77); 79 (75)
10	17.46	Maleic acid TMS derivative	147 (999); 73 (934); 75 (257); 148 (161); 245 (97); 149 (90); 83 (89); 74 (83); 58 (71); 59 (66)
11	17.75	Succinic acid TMS derivative	147 (999); 73 (721); 75 (337); 148 (153); 55 (140); 247 (99); 149 (84); 129 (80); 56 (72); 74 (72)
12	18.11	Glyceric acid TMS derivative	73 (999); 147 (392); 189 (196); 133 (167); 103 (154); 75 (112); 292 (106); 74 (91); 59 (78); 117 (78)
13	18.50	Itaconic acid TMS derivative	147 (999); 73 (986); 75 (308); 148 (156); 74 (101); 149 (94); 259 (77); 67 (65); 72 (63); 97 (59)
14	18.73	Fumaric acid TMS derivative	245 (999); 73 (981); 75 (595); 147 (545); 57 (362); 143 (219); 83 (208); 246 (187); 115 (153); 55 (143)
15	20.03	Mesaconic acid TMS derivative	73 (999); 147 (440); 75 (351); 184 (295); 259 (224); 67 (113); 74 (95); 97 (94); 58 (62); 148 (62)
16	20.63	Aspartic acid TMS derivative	73 (999); 160 (412); 75 (325); 130 (206); 117 (154); 116 (147); 147 (128); 100 (123); 74 (113); 161 (54)
17	22.20	Malic acid TMS derivative	73 (999); 147 (392); 75 (112); 233 (111); 133 (90); 55 (89); 74 (85); 148 (61); 245 (57); 101 (52)
18	22.62	Erythritol TMS derivative	73 (999); 147 (311); 217 (248); 103 (199); 205 (147); 117 (136); 75 (122); 204 (86); 74 (85); 115 (80)
19	22.71	Salicylic acid TMS derivative	73 (999); 267 (464); 75 (133); 135 (130); 268 (114); 91 (92); 74 (87); 147 (72); 149 (59); 269 (49)
20	23.03	5-oxoproline TMS derivative	156 (999); 73 (794); 147 (171); 75 (165); 157 (122); 74 (84); 59 (70); 230 (67); 87 (60); 258 (60)
21	23.17	GABA TMS derivative	174 (999); 73 (832); 147 (261); 75 (187); 175 (182); 304 (149); 59 (137); 86 (103); 74 (78); 176 (78)
22	23.47	Threonic acid TMS derivative	73 (999); 147 (315); 292 (161); 75 (129); 220 (113); 205 (103); 103 (96); 117 (95); 74 (88); 217 (65)
23	23.70	Phenylalanine TMS derivative	120 (999); 73 (488); 75 (391); 146 (383); 91 (209); 130 (170); 74 (112); 103 (100); 121 (97); 77 (92)
24	26.46	Asparagine TMS derivative	73 (999); 116 (459); 75 (252); 132 (212); 231 (199); 147 (152); 74 (134); 100 (113); 188 (108); 141 (77)
25	26.15	3.4.5-Trihydroxypentanoic acid TMS derivative	73 (999); 147 (241); 75 (173); 217 (91); 74 (83); 129 (77); 205 (77); 117 (70); 133 (70); 204 (64)
26	27.89	NI-1	73 (999); 147 (350); 205 (217); 217 (127); 117 (97); 75 (93); 74 (87); 103 (68); 148 (60); 133 (58)
27	28.22	Aconitic acid TMS derivative	73 (999); 147 (352); 231 (241); 75 (169); 229 (96); 74 (90); 133 (82); 148 (62); 67 (52); 149 (51)

Peak	R.T. (min)	Constituent	Experimental main fragments: *m/z* (%)
28	28.40	Ribonic acid TMS derivative	73 (999); 147 (268); 103 (183); 292 (170); 217 (168); 75 (126); 74 (95); 117 (64); 293 (50); 133 (48)
29	28.77	NI-2	73 (999); 147 (275); 103 (189); 292 (175); 75 (157); 217 (113); 74 (88); 205 (59); 117 (56); 133 (53)
30	29.25	Azelaic acid TMS derivative	73 (999); 75 (572); 147 (201); 55 (196); 117 (184); 217 (139); 129 (138); 74 (103); 201 (86); 317 (81)
31	29.62	Citric acid TMS derivative	73 (999); 147 (367); 273 (234); 75 (133); 74 (83); 149 (59); 133 (58); 148 (57); 274 (53); 347 (37)
32	30.35	NI-3	73 (999); 147 (293); 345 (219); 255 (119); 75 (103); 74 (77); 346 (69); 204 (65); 133 (56); 191 (48)
33	30.63	Fructose TMS/MeOX derivative	73 (999); 103 (547); 217 (243); 147 (221); 307 (138); 74 (87); 133 (83); 117 (60); 75 (56); 104 (51)
34	31.13	Glucose TMS/MeOX derivative	73 (999); 205 (317); 147 (284); 319 (254); 160 (208); 103 (132); 217 (112); 74 (88); 117 (77); 320 (75)
35	31.43	Galactose TMS/MeOX derivative	73 (999); 147 (225); 205 (206); 103 (147); 75 (139); 319 (122); 217 (114); 74 (89); 160 (80); 117 (67)
36	31.75	Sorbitol TMS derivative	73 (999); 147 (307); 205 (250); 103 (189); 319 (186); 217 (165); 117 (96); 74 (85); 75 (80); 204 (60)
37	32.18	*p*-Coumaric acid TMS derivative	73 (999); 75 (370); 219 (298); 293 (204); 308 (159); 147 (116); 249 (112); 74 (95); 59 (73); 179 (69)
38	32.40	Gallic acid TMS derivative	73 (999); 281 (203); 458 (85); 74 (81); 179 (69); 147 (67); 75 (65); 282 (48); 133 (42); 459 (37)
39	33.13	Gluconic acid TMS derivative	73 (999); 147 (279); 75 (115); 103 (113); 292 (99); 74 (85); 217 (81); 205 (79); 333 (74); 117 (52)
40	33.33	(3-Hydroxy-4-methoxyphenyl)ethylene glycol TMS derivative	73 (999); 297 (684); 298 (174); 147 (162); 75 (158); 74 (88); 299 (79); 209 (69); 59 (36); 133 (35)
41	34.83	*Myo*-Inositol TMS derivative	73 (999); 147 (382); 217 (346); 305 (200); 191 (192); 318 (112); 204 (96); 74 (94); 75 (88); 133 (81)
42	38.75	Methyl galactoside TMS derivative	73 (999); 204 (634); 75 (327); 147 (287); 103 (187); 217 (186); 205 (148); 129 (102); 74 (89); 56 (72)
43	43.76	Sucrose TMS derivative	73 (999); 217 (374); 361 (368); 147 (264); 103 (210); 129 (138); 362 (115); 218 (89); 74 (85); 169 (83)
44	12.70	NI-4	68 (999); 57 (740); 95 (722); 82 (720); 55 (715); 69 (647); 67 (520); 81 (482); 83 (436); 123 (402)
45	12.96	Myristic acid TMS derivative	73 (999); 75 (745); 117 (704); 285 (394); 129 (350); 132 (299); 55 (263); 145 (165); 69 (144); 74 (108)
46	13.61	NI-5	82 (999); 95 (693); 57 (622); 81 (608); 55 (458); 67 (390); 68 (369); 71 (321); 69 (309); 123 (307)
47	16.80	NI-6	73 (999); 75 (142); 74 (87); 117 (51); 55 (48); 54 (33); 143 (30); 311 (27); 57 (23); 67 (22)
48	16.98	Palmitic acid TMS derivative	73 (999); 117 (922); 75 (733); 313 (463); 129 (413); 132 (390); 55 (275); 145 (250); 57 (143); 314 (113)
49	18.82	Margaric acid TMS derivative	73 (999); 117 (859); 75 (718); 129 (391); 132 (390); 327 (389); 55 (310); 145 (258); 57 (177); 69 (145)
50	19.44	Phytol TMS derivative	143 (999); 73 (327); 75 (262); 144 (127); 123 (123); 57 (103); 55 (94); 81 (90); 69 (64); 95 (47)
51	20.00	Linoleic acid TMS derivative	73 (999); 75 (971); 67 (651); 81 (512); 55 (498); 79 (333); 95 (326); 129 (324); 117 (246); 54 (236)
52	20.11	Linolenic acid TMS derivative	75 (999); 73 (973); 79 (714); 67 (492); 55 (441); 95 (421); 93 (348); 129 (346); 117 (317); 81 (313)
53	20.58	Stearic acid TMS derivative	73 (999); 117 (891); 75 (703); 132 (415); 129 (403); 341 (390); 55 (335); 145 (286); 57 (226); 69 (161)
54	23.69	NI-7	73 (999); 75 (396); 183 (271); 369 (197); 79 (121); 55 (105); 74 (101); 93 (99); 129 (95); 147 (95)
55	23.86	Arachidic acid TMS derivative	73 (999); 117 (734); 75 (666); 129 (371); 132 (354); 55 (331); 369 (264); 57 (256); 145 (244); 69 (175)
56	24.20	NI-8	73 (999); 173 (507); 75 (378); 55 (139); 129 (126); 103 (123); 81 (115); 67 (99); 74 (98); 357 (98)

Peak	R.T. (min)	Constituent	Experimental main fragments: *m/z* (%)
57	24.27	NI-9	73 (999); 171 (684); 75 (385); 81 (237); 129 (207); 103 (124); 340 (119); 79 (109); 172 (105); 55 (99)
58	26.29	Monopalmitin TMS derivative	73 (999); 131 (591); 371 (414); 75 (297); 147 (285); 57 (275); 55 (214); 129 (184); 71 (136); 372 (126)
59	26.89	Behenic acid TMS derivative	73 (999); 117 (833); 75 (626); 132 (420); 129 (413); 55 (330); 145 (330); 57 (319); 397 (266); 69 (175)
60	27.71	Heptacosane	57 (999); 71 (616); 85 (419); 55 (266); 69 (166); 56 (144); 99 (117); 83 (107); 70 (103); 97 (85)
61	28.33	NI-10	73 (999); 117 (888); 75 (714); 132 (463); 129 (439); 55 (400); 57 (383); 145 (381); 411 (277); 69 (205)
62	28.74	2-Monostearin TMS derivative	73 (999); 129 (509); 55 (418); 147 (390); 67 (329); 75 (295); 103 (294); 81 (283); 57 (279); 69 (248)
63	29.71	Lignoceric acid TMS derivative	73 (999); 117 (861); 75 (625); 132 (470); 129 (423); 145 (391); 57 (383); 55 (371); 425 (249); 69 (203)
64	30.50	Hentriacontane	57 (999); 71 (645); 85 (420); 55 (265); 69 (175); 56 (146); 83 (131); 99 (121); 70 (105); 97 (100)
65	31.05	Hyenic acid TMS derivative	73 (999); 117 (830); 75 (633); 132 (430); 57 (425); 129 (406); 55 (402); 145 (363); 69 (251); 439 (229)
66	31.16	1-hexacosanol	75 (999); 57 (697); 439 (685); 73 (643); 55 (437); 69 (394); 103 (367); 71 (359); 83 (306); 440 (256)
67	31.46	NI-11	57 (999); 152 (794); 55 (636); 69 (533); 96 (494); 82 (445); 97 (429); 83 (407); 81 (395); 117 (372)
68	32.35	Ceric acid TMS derivative	73 (999); 117 (875); 57 (872); 55 (662); 75 (626); 132 (488); 129 (417); 145 (417); 69 (406); 83 (352)
69	32.44	NI-12	57 (999); 152 (679); 55 (640); 69 (496); 75 (465); 73 (412); 83 (408); 97 (398); 96 (379); 71 (373)
70	33.10	Tetratetracontane	57 (999); 71 (657); 85 (441); 55 (294); 69 (215); 83 (146); 56 (142); 97 (122); 73 (119); 99 (118)
71	33.72	Octacosyl TMS derivative	75 (999); 57 (716); 467 (670); 73 (525); 55 (420); 103 (401); 69 (342); 71 (333); 83 (314); 97 (274)
72	34.00	NI-13	57 (999); 152 (733); 55 (565); 96 (501); 69 (458); 82 (445); 83 (412); 97 (383); 81 (356); 71 (344)
73	34.30	α-Tocopherol acetate	165 (999); 430 (716); 57 (450); 207 (404); 55 (313); 164 (301); 73 (230); 431 (225); 69 (211); 71 (181)
74	34.50	NI-14	73 (999); 69 (800); 55 (705); 57 (691); 207 (474); 81 (407); 83 (359); 75 (337); 152 (323); 71 (309)
75	34.84	NI-15	73 (999); 117 (904); 57 (624); 75 (617); 132 (525); 55 (475); 145 (464); 129 (451); 69 (301); 71 (242)
76	34.90	NI-16	57 (999); 55 (636); 82 (465); 69 (461); 83 (455); 71 (443); 73 (429); 75 (429); 97 (386); 96 (381)
77	35.15	Campesterol TMS derivative	73 (999); 129 (946); 75 (556); 55 (501); 57 (493); 71 (386); 95 (383); 81 (382); 69 (343); 343 (319)
78	35.54	Stigmasterol TMS derivative	83 (999); 55 (856); 73 (694); 69 (605); 129 (596); 57 (492); 81 (453); 75 (413); 71 (278); 95 (244)
79	35.67	NI-17	73 (999); 55 (637); 116 (620); 75 (532); 69 (522); 207 (474); 57 (436); 117 (355); 81 (352); 83 (338)
80	35.78	NI-18	73 (999); 484 (447); 207 (375); 75 (374); 55 (338); 57 (338); 69 (303); 81 (227); 129 (211); 95 (196)
81	35.87	NI-19	218 (999); 203 (598); 55 (487); 69 (424); 73 (366); 81 (298); 95 (296); 93 (222); 91 (219); 105 (219)
82	36.08	1-triacontanol TMS derivative	75 (999); 57 (796); 73 (618); 495 (566); 55 (469); 103 (412); 69 (392); 71 (386); 83 (329); 97 (272)
83	36.24	β-Sitosterol TMS derivative	129 (999); 73 (747); 57 (511); 75 (488); 55 (423); 95 (376); 81 (361); 119 (305); 107 (303); 105 (296)
84	36.33	NI-20	218 (999); 73 (752); 55 (461); 203 (415); 95 (393); 69 (386); 75 (383); 81 (336); 124 (300); 93 (252)
85	36.40	NI-21	55 (999); 69 (947); 73 (947); 57 (725); 218 (615); 95 (592); 81 (578); 109 (431); 67 (411); 93 (379)
86	36.56	NI-22	73 (999); 134 (899); 129 (750); 95 (523); 69 (499); 55 (440); 109 (407); 81 (376); 75 (371); 119 (366)
87	36.73	NI-23	55 (999); 69 (490); 81 (437); 73 (409); 83 (365); 97 (329); 79 (324); 67 (312); 95 (302); 91 (287)

Peak	R.T. (min)	Constituent	Experimental main fragments: *m/z* (%)
88	36.87	NI-24	69 (999); 73 (965); 55 (546); 75 (424); 81 (408); 95 (384); 109 (337); 207 (294); 91 (264); 67 (260)
89	37.04	NI-25	73 (999); 55 (541); 207 (411); 57 (382); 69 (365); 484 (356); 81 (282); 95 (261); 75 (246); 67 (223)
90	37.17	NI-26	73 (999); 57 (749); 117 (728); 55 (646); 75 (506); 69 (455); 132 (414); 145 (398); 129 (368); 71 (318)
91	37.28	NI-27	73 (999); 55 (495); 69 (446); 207 (433); 75 (414); 95 (355); 81 (334); 57 (324); 135 (257); 109 (238)
92	37.42	β-sitostenone	124 (999); 55 (500); 57 (359); 229 (325); 95 (294); 79 (277); 91 (266); 69 (259); 81 (254); 93 (246)
93	37.53	NI-28	134 (999); 73 (456); 129 (387); 135 (234); 95 (229); 119 (224); 75 (194); 55 (192); 69 (178); 93 (178)
94	37.79	NI-29	73 (999); 55 (502); 207 (403); 75 (378); 69 (359); 95 (347); 57 (295); 81 (292); 107 (248); 67 (238)
95	37.99	NI-30	73 (999); 144 (912); 207 (617); 55 (408); 75 (353); 69 (346); 95 (314); 81 (290); 135 (275); 145 (243)
96	38.27	Friedelin	69 (999); 95 (835); 55 (828); 81 (692); 109 (645); 67 (585); 123 (580); 96 (520); 125 (453); 207 (393)
97	38.34	NI-31	73 (999); 75 (587); 207 (585); 57 (526); 55 (400); 69 (364); 71 (251); 83 (241); 103 (207); 281 (204)
98	38.66	NI-32	73 (999); 207 (939); 55 (367); 69 (353); 135 (345); 95 (300); 281 (286); 81 (282); 147 (272); 119 (238)
99	38.84	NI-33	73 (999); 207 (747); 55 (408); 135 (401); 69 (348); 81 (264); 281 (257); 75 (256); 95 (241); 147 (226)
100	39.21	NI-34	73 (999); 207 (668); 55 (294); 75 (273); 69 (252); 135 (242); 57 (235); 281 (233); 147 (190); 81 (187)
101	39.53	NI-35	73 (999); 207 (533); 75 (279); 129 (252); 55 (249); 57 (226); 135 (209); 147 (178); 281 (173); 69 (166)
102	39.59	NI-36	73 (999); 207 (513); 247 (424); 55 (252); 57 (213); 248 (198); 75 (192); 69 (174); 135 (167); 281 (152)

Table S.1.4 – Molecular weight and molecular formula of the constituents detected by HPLC-ESIMS/MS in the hydroethanol and aqueous extracts of leaves from all Brazilian bamboo studied.

Peak	Adduct	R.T. (min)	Constituent	Molecular masst	Molecular formula	Compound class
103	M+H	4.42	Deoxyfructosazine	304.3	$C_{12}H_{20}N_2O_7$	Pyrazine derivative
104	M+H	6.56	Hyperoside	464.4	$C_{21}H_{20}O_{12}$	Flavonol
105	M+H	7.82	Cordycepin	251.2	$C_{10}H_{13}N_5O_3$	Nucleoside derivative
106	M+H	8.02	Inosine	268.2	$C_{10}H_{12}N_4O_5$	Nucleoside
107	M+H	14.52	Succinoadenosine	383.3	$C_{14}H_{17}N_5O_8$	Nucleoside derivative
108	$[M-H]^-$	15.73	Isochlorogenic acid b	516.4	$C_{25}H_{24}O_{12}$	Hydroxycinnamic acid derivative
109	M+H	15.95	Tryptophan N-glucoside	366.4	$C_{17}H_{22}N_2O_7$	Amino acid
110	$[M-H]^-$	16.25	(3R,5S)-4-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,3,5-trihydroxycyclohexane-1-carboxylic acid	354.3	$C_{16}H_{18}O_9$	Hydroxycinnamic acid derivative
111	M+H	16.35	Chlorogenic acid	354.3	$C_{16}H_{18}O_9$	Hydroxycinnamic acid derivative
112	M+H	16.44	4-*p*-Coumaroylquinic acid	338.3	$C_{16}H_{18}O_8$	Hydroxycinnamic acid derivative
113	M+H	16.87	L-Tryptophan	204.2	$C_{11}H_{12}N_2O_2$	Amino acid
114	M+H	18.26	Kaempferol 3-glucuronide	462.4	$C_{21}H_{18}O_{12}$	Flavonol
115	M+H	18.42	Luteolin 7-glucuronide	462.4	$C_{21}H_{18}O_{12}$	Flavone
116	M+H	19.62	Licoagroside B	432.4	$C_{18}H_{24}O_{12}$	Soluble sugar derivative
117	M+H	20.94	Riboflavin	376.4	$C_{17}H_{20}N_4O_6$	Vitamin
118	$[M-H]^-$	22.14	Apigenin 6,8-digalactoside	594.5	$C_{27}H_{30}O_{15}$	Flavone
119	M+H; $[M-H]^-$	23.71	Arillatose B	518.5	$C_{22}H_{30}O_{14}$	Soluble sugar derivative
120	M+H	23.96	Vicenin	594.5	$C_{27}H_{30}O_{15}$	Flavone
121	$[M-H]^-$	25.70	Sibiricose A_1	548.5	$C_{23}H_{32}O_{15}$	Hydroxycinnamic acid derivative
122	$[M-H]^-$	25.71	Corymboside	564.5	$C_{26}H_{28}O_{14}$	Flavone
123	M+H	26.47	(E)-N-(4-acetamidobutyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide	306.3	$C_{16}H_{22}N_2O_4$	Phenylamide

Peak	Adduct	R.T. (min)	Constituent	Molecular masst	Molecular formula	Compound class
124	M+H	27.39	5,7-dihydroxy-2-phenyl-6.8-bis[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one	578.5	$C_{27}H_{30}O_{14}$	Flavonol
125	M-H	28.11	Saponarin	594.5	$C_{27}H_{30}O_{15}$	Flavone
126	M+H/[M-H]$^-$	29.14	Schaftoside	564.5	$C_{26}H_{28}O_{14}$	Flavone
127	[M-H]-	29.75	Vitexin -4"-*O*-glucoside	594.5	$C_{27}H_{30}O_{15}$	Flavone
128	M+H	29.77	8-[4,5-dihydroxy-6-(hydroxymethyl)-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]-5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one	594.5	$C_{27}H_{30}O_{15}$	Flavone
129	M+H	30.24	2"-*O*-beta-D-Xylopyranosylorientin	580.5	$C_{26}H_{28}O_{15}$	Flavone
130	M-H	30.91	Swertisin	446.4	$C_{22}H_{22}O_{10}$	Flavone
131	[M+H]	30.92	Isoorientin	448.4	$C_{21}H_{20}O_{11}$	Flavone
132	M+H/[M-H]$^-$	31.42	Tricin 5-glucoside	492.4	$C_{23}H_{24}O_{12}$	Flavone
133	[M-H]$^-$	32.26	Violanthin	578.5	$C_{27}H_{30}O_{14}$	Flavone
134	M+H	32.41	Isovitexin	432.4	$C_{21}H_{20}O_{10}$	Flavone
135	M+H	32.69	Quercetin 3,4'-*O*-diglucoside	626.5	$C_{27}H_{30}O_{17}$	Flavonol
136	M+H	33.03	Isovitexin 2"-*O*-arabinoside	564.5	$C_{26}H_{28}O_{14}$	Flavone
137	M+H	33.61	Syringetin-3-*O*-glucoside	508.4	$C_{23}H_{24}O_{13}$	Flavonol
138	M+H	33.73	*C*-Hexosyl-chrysoeriol *O*-hexoside	624.2	$C_{28}H_{32}O_{16}$	Flavone
139	M+H	33.87	6-hydroxy-3-[3-hydroxy-4-[3,4,5-trihydroxy-6-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyphenyl]-5,7-dimethoxychromen-4-one	654.6	$C_{29}H_{34}O_{17}$	Flavonol
140	M-H/M+H	33.87	Vitexin	432.4	$C_{21}H_{20}O_{10}$	Flavone
141	M-H	33.96	Afzelin	432.4	$C_{21}H_{20}O_{10}$	Flavone
142	[M-H]$^-$	34.02	6-*O*-Caffeoylarbutin	434.4	$C_{21}H_{22}O_{10}$	Hydroxycinnamic acid derivative
143	M+H	34.48	5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-bis(3,4,5-trihydroxyoxan-2-yl)chromen-4-one	534.5	$C_{25}H_{26}O_{13}$	Flavone
144	M+H	34.88	*C*-Hexosyl-chrysoeriol *O*-pentoside	594.2	$C_{27}H_{30}O_{15}$	Flavone
145	[M-H]$^-$	36.07	(2*Z*)-4,6-dihydroxy-2-[(4-hydroxy-3,5-dimethoxyphenyl)methylidene]-1-benzofuran-3-one	330.3	$C_{17}H_{14}O_7$	Aurone
146	M+H	37.73	Isoscoparin	462.4	$C_{22}H_{22}O_{11}$	Flavone

Peak	Adduct	R.T. (min)	Constituent	Molecular masst	Molecular formula	Compound class
147	M+H/[M-H]$^-$	39.49	Rhamnetin 3-sophoroside	640.5	$C_{28}H_{32}O_{17}$	Flavonol
148	Cat	40.39	Peonidin-3-glucoside	463.4	$C_{22}H_{23}O_{11}$	Anthocyanidin
149	M+H	40.45	Chrysoeriol	300.3	$C_{16}H_{12}O_6$	Flavone
150	M+H	40.52	*C*-Hexosyl-chrysoeriol	462.1	$C_{22}H_{22}O_{11}$	Flavone
151	M+H	40.52	Peonidin 3-galactoside	498.9	$C_{22}H_{23}ClO_{11}$	Anthocyanidin
152	[M-H]$^-$	40.61	Liriodendrin	742.7	$C_{34}H_{46}O_{18}$	Lignan
153	M+H	44.46	Feruloyltyramine	313.3	$C_{18}H_{19}NO_4$	Alkaloid
154	M+H	44.68	5-hydroxy-7-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2-[4-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyphenyl]chromen-4-one	578.5	$C_{27}H_{30}O_{14}$	Flavone
155	M+H	45.54	Rhoifolin	578.5	$C_{27}H_{30}O_{14}$	Flavone
156	M+H	45.55	3-(alpha-L-Rhamnopyranosyloxy)-8-(beta-D-glucopyranosyloxy)-3',4',5-trihydroxy-7-methoxyflavone	640.5	$C_{28}H_{32}O_{17}$	Flavone
157	[M-H]$^-$	45.65	Diosmin	608.5	$C_{28}H_{32}O_{15}$	Flavone
158	M+H	45.66	Neodiosmin	608.5	$C_{28}H_{32}O_{15}$	Flavone
159	M+H	45.73	Chrysoeriol 7-*O*-Neohesperidoside	608.5	$C_{28}H_{32}O_{15}$	Flavone
160	[M+H]	45.73	Apigetrin	432.4	$C_{21}H_{20}O_{10}$	Flavone
161	M+H	45.92	Apigenin 4'-*O*-glucoside	432.4	$C_{21}H_{20}O_{10}$	Flavone
162	M+H	46.18	Matairesinol	358.4	$C_{20}H_{22}O_6$	Lignan
163	M+H	46.24	Pectolinarin	622.6	$C_{29}H_{34}O_{15}$	Flavonol
164	[M-H]$^-$	46.32	6-hydroxy-3-[3-hydroxy-4-[3,4,5-trihydroxy-6-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyphenyl]-5,7-dimethoxychromen-4-one	654.6	$C_{29}H_{34}O_{17}$	Flavonol
165	M+H	46.40	3-[(2S,3R,4S,5S,6R)-4.5-dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxychromen-4-one	624.5	$C_{28}H_{32}O_{16}$	Flavonol
166	M+H	47.19	Afromosin 7-*O*-glucoside	460.4	$C_{23}H_{24}O_{10}$	Isoflavone
167	M-H	47.21	Tricin 7-glucoside	492.4	$C_{23}H_{24}O_{12}$	Flavone
168	M+H	47.37	Spermidine-diferuloyl			Phenylamide

Peak	Adduct	R.T. (min)	Constituent	Molecular masst	Molecular formula	Compound class
169	M-H	47.63	Tricin 7-glucuronide	506.4	$C_{23}H_{22}O_{13}$	Flavone
170	M+H	54.23	Spinacetin 3-gentiobioside	670.6	$C_{29}H_{34}O_{18}$	Flavonol
171	M+H	54.63	4-*O*-Acetyl-beta-D-fructofuranosyl 6-*O*-[(2E)-3-phenyl-2-propenoyl]-alpha-D-glucopyranoside	514.5	$C_{23}H_{30}O_{13}$	Phenylglycoside
172	M+H	56.13	Costunolide	232.3	$C_{15}H_{20}O_2$	Sesquiterpene lactone
173	M+H	60.04	3-*p*-coumaroylquinic acid	338.3	$C_{16}H_{18}O_8$	Hydroxycinnamic acid derivative
174	M+H	63.08	Apaensin	316.3	$C_{17}H_{16}O_6$	Chromen
175	M+H	65.62	Adenosine 5'-diphosphate	427.2	$C_{10}H_{15}N_5O_{10}P_2$	Nucleotide derivative
176	M+H	67.45	Monolinolenin	352.5	$C_{21}H_{36}O_4$	Glycerides
177	M+H	68.31	Methoxy-myricetin-*O*-hexosyl-*O*-hexoside	0		Flavone
178	M+H	69.87	2-Linoleoylglycerol	354.5	$C_{21}H_{38}O_4$	Glycerides
179	M-H	69.92	Glc-Glc-octadecatrienoyl-sn-glycerol	676.4	$C_{33}H_{56}O_{14}$	Glycerides
180	M-H	70.59	1-Hexadecanoyl-sn-glycero-3-phospho-(1'-myo-inositol)	572.6	$C_{25}H_{49}O_{12}P$	Glycerophosphoinositols
181	$[M-H]^-$	76.30	2',5,6-trimethoxyflavone	312.3	$C_{18}H_{16}O_5$	Flavone
182	$[M-H]^-$	78.59	Docosanol	326.6	$C_{22}H_{46}O$	Aliphatic alcohol
183	M+H	78.65	1-(2E,4E-octadecadienoyl)-sn-glycero-3-phosphocholine	519.7	$C_{26}H_{50}NO_7P$	Glycerophospholipids
184	M-H	80.09	1-hexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)	484.6	$C_{22}H_{45}O_9P$	Glycerophospholipids
185	$[M-H]^-$	80.10	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol	749	$C_{40}H_{77}O_{10}P$	Glycerophospholipids
186	$[M-H]^-$	80.12	(8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl) (*Z*)-2-methylbut-2-enoate	342.3	$C_{19}H_{18}O_6$	Chromen
187	M+H	80.33	2-Palmitoyl-sn-glycero-3-phosphocholine	495.6	$C_{24}H_{50}NO_7P$	Glycerophospholipids
188	M-H	80.89	1-(9*Z*-octadecenoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)	510.6	$C_{24}H_{47}O_9P$	Glycerophospholipids
189	M+H	81.13	2-Oleoyl-sn-glycero-3-phosphocholine	521.7	$C_{26}H_{52}NO_7P$	Glycerophospholipids
190	M+H	81.20	2-octadecanoyl-sn-glycero-3-phosphocholine	523.7	$C_{26}H_{54}NO_7P$	Glycerophospholipids
191	$[M-H]^-$	81.66	Juniperoside III	312.31	$C_{15}H_{20}O_7$	Phenylglycoside
192	M+H	81.70	Polanrazine B	377.5	$C_{18}H_{23}N_3O_2S_2$	Pyrazine derivative
193	M+H	82.18	1,2-dia-linolenoyl-rac-glycerol	612.9	$C_{39}H_{64}O_5$	Glycerides
194	M+H	83.53	Enniatin B	639.8	$C_{33}H_{57}N_3O_9$	Cyclic depsihexapeptides
195	M-H	83.95	1-hexadecanoyl-2-(9*Z*,12*Z*-octadecadienoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)	747	$C_{40}H_{75}O_{10}P$	Glycerophospholipids

Peak	Adduct	R.T. (min)	Constituent	Molecular masst	Molecular formula	Compound class
196	M+H	84.00	1-Stearoyl-2-linoleoylphosphatidylcholine	786.1	$C_{44}H_{84}NO_8P$	Glycerophospholipids
197	M+H	84.44	Campesterol	400.7	$C_{28}H_{48}O$	Phytosterol
198	M+H	85.03	*β*-Sitosterol	414.7	$C_{29}H_{50}O$	Phytosterol
199	M+H	85.99	Avobenzone	310.4	$C_{20}H_{22}O_3$	Dihydrochalcones
200	M+H	86.26	1-hexadecanoyl-2-(9*Z*,12*Z*,15*Z*-octadecatrienoyl)-sn-glycerol	590.9	$C_{37}H_{66}O_5$	Glycerides
201	M+H	86.67	Galactosylceramide (d18:2/18:1)		$C_{42}H_{77}NO_8$	Sphingolipids
202	M+H	87.25	1-palmitoyl-2-homo-g-linolenoyl-sn-glycero-3-phosphocholine	784.1	$C_{44}H_{82}NO_8P$	Glycerophospholipids
203	M+H	87.65	N-(11*Z*-eicosenoyl)-1-*β*-galactosyl-4E,14*Z*-sphingadienine	752.1	$C_{44}H_{81}NO_8$	Sphingolipids
204	M+H	87.67	N-(11*Z*-eicosenoyl)-4E,14*Z*-sphingadienine	590	$C_{38}H_{71}NO_3$	Sphingolipids
205	M+H	88.07	Eudesmin	386.4	$C_{22}H_{26}O_6$	Lignan
206	M+H	88.65	Galactosylceramide (d18:2/22:1)		$C_{46}H_{85}NO_8$	Sphingolipids
207	M+H	90.31	Chiococcasaponin IV	909.1	$C_{47}H_{72}O_{17}$	Saponin
208	M+H	90.73	N-(octadecanoyl)-sphinganine	568	$C_{36}H_{73}NO_3$	Sphingolipids
209	M+H	91.05	Koaburside	346.3	$C_{15}H_{22}O_9$	Phenylglycoside
210	M+H	91.07	Avocadene 4-acetate	328.5	$C_{19}H_{36}O_4$	Acetogenins
211	M+H	91.13	1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine	760.1	$C_{42}H_{82}NO_8P$	Glycerophospholipids
212	M+H	91.22	Isosulochrin	332.3	$C_{17}H_{16}O_7$	Anthraquinone
213	M+H	91.25	*α*-tochopheryl acetate	472.7	$C_{31}H_{52}O_3$	Vitamin
214	M+H	91.27	Sarmentoside B	664.7	$C_{34}H_{48}O_{13}$	Cardenolide
215	M+H	92.06	Bidwillon A	408.5	$C_{25}H_{28}O_5$	Isoflavone
216	M+H	93.31	Pheophytin	871.2	$C_{55}H_{74}N_4O_5$	Pigment
217	M+H	93.83	Sclareol	308.5	$C_{20}H_{36}O_2$	Diterpenoid

Suplementary data: Chapter 2

Table S.2.1 – Standard curves used to calculate the EC_{50} and IC_{50} of the standards and samples from six Brazilian bamboo species in the antioxidants and anticholinesterase assays. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata*; **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora*. The final letter in each acronym of the bamboo species corresponds to the type of extract**: (E)** Hydroethanol extract, **(A)** Aqueous extract, **(H)** Hexane extract. (*) not calculated.

Assay	Sample	Concentration	Line equation	R^2
DPPH	OGA	1.1 µg/mL - 90.9 µg/mL	y= 0.1506x + 1.0207	0.9451
	PMA	1.1 µg/mL - 90.9 µg/mL	y= 0.4105x + 1.2101	0.9835
	FAA	1.1 µg/mL - 90.9 µg/mL	y= 0.3191x + 2.0707	0.9952
	FCA	1.1 µg/mL - 90.9 µg/mL	y= 0.2717x + 3.5299	0.9884
	AAA	1.1 µg/mL - 90.9 µg/mL	y= 0.2174x + 3.1766	0.9964
	OGE	1.1 µg/mL - 90.9 µg/mL	y= 0.0865x + 2.846	0.9987
	PME	1.1 µg/mL - 90.9 µg/mL	y= 0.4616x + 2.7474	0.9988
	FAE	1.1 µg/mL - 90.9 µg/mL	y= 0.4481x + 5.4359	0.9969
	FCE	1.1 µg/mL - 90.9 µg/mL	y= 0.36x + 8.266	0.9938
	AAE	1.1 µg/mL - 90.9 µg/mL	y= 0.2091x + 6.4918	0.9972
	OGH	1.1 µg/mL - 90.9 µg/mL	y= 0.0197x + 3.31	0.9749
	PMH	1.1 µg/mL - 90.9 µg/mL	y= 0.0684x + 4.5728	0.9952
	FAH	1.1 µg/mL - 90.9 µg/mL	y= 0.0136x + 10.735	0.9214
	FCH	1.1 µg/mL - 90.9 µg/mL	*	*
	AAH	1.1 µg/mL - 90.9 µg/mL	*	*
	Trolox	12.5 µg/mL - 200 µg/mL	y= 0.531x - 0.2745	0.9964
	Quercetin	15 µg/mL - 240 µg/mL	y= 0.3677x + 4.7993	0.9968
ORAC	OGA	3.9 µg/mL - 250 µg/mL	y= 0.4634x + 0.2449	0.9924
	PMA	3.9 µg/mL - 250 µg/mL	y= 1.3479x + 0.8053	0.9995
	FAA	3.9 µg/mL - 250 µg/mL	y= 1.2379x + 2.0076	0.9891
	FCA	3.9 µg/mL - 250 µg/mL	y= 1.0732x + 1.3151	0.9937
	AAA	3.9 µg/mL - 250 µg/mL	y= 0.6424x + 4.5363	0.9834
	OGE	3.9 µg/mL - 250 µg/mL	y= 0.6217x + 0.9676	0.9862
	PME	3.9 µg/mL - 250 µg/mL	y= 2.4019x - 2.9253	0.9833
	FAE	3.9 µg/mL - 250 µg/mL	y= 1.5187x + 3.444	0.974
	FCE	3.9 µg/mL - 250 µg/mL	y= 1.982x - 0.3726	0.9995
	AAE	3.9 µg/mL - 250 µg/mL	y= 0.957x + 1.6098	0.9896
	OGH	3.9 µg/mL - 250 µg/mL	y= 0.1823x + 0.2588	0.9877
	PMH	3.9 µg/mL - 250 µg/mL	y= 0.109x + 0.6659	0.9967
	FAH	3.9 µg/mL - 250 µg/mL	y= 0.0675x + 0.9168	0.9678
	FCH	3.9 µg/mL - 250 µg/mL	y= 0.0173x + 2.8524	0.9421
	AAH	3.9 µg/mL - 250 µg/mL	y= 0.107x + 0.9429	0.9912
	Trolox	0.03 µg/mL - 0.5 µg/mL	y= 218.23x - 1.6262	0.997

Assay	Sample	Concentration	Line equation	R^2
AChE	OGA	3.1 µg/mL - 200 µg/mL	y=15.63ln(x)-52.318	0.95
	PMA	3.1 µg/mL - 200 µg/mL	y=10.847ln(x)-15.652	0.9288
	FAA	3.1 µg/mL - 200 µg/mL	y=9.2516ln(x)-12.654	0.9657
	FCA	3.1 µg/mL - 200 µg/mL	y=18.517ln(x)-68.741	0.993
	AAA	3.1 µg/mL - 200 µg/mL	y=5.049ln(x)+11.397	0.9866
	MPA	3.1 µg/mL - 200 µg/mL	y=9.1164ln(x)-5.1355	0.9778
	OGE	3.1 µg/mL - 200 µg/mL	y=13.72ln(x)-44.913	0.9373
	PME	3.1 µg/mL - 200 µg/mL	y=5.7383ln(x)-6.0323	0.9275
	FAE	3.1 µg/mL - 200 µg/mL	y=6.7331ln(x)-2.5367	0.9673
	FCE	3.1 µg/mL - 200 µg/mL	y=12.771ln(x)-31.012	0.9485
	AAE	3.1 µg/mL - 200 µg/mL	y=8.562ln(x)-2.0973	0.9724
	MPE	3.1 µg/mL - 200 µg/mL	y=13.721ln(x)-32.247	0.9449
	OGH	3.1 µg/mL - 200 µg/mL	y=8.4181ln(x)-18.687	0.9625
	PMH	3.1 µg/mL - 200 µg/mL	y=6.4589ln(x)-1.6451	0.9612
	FAH	3.1 µg/mL - 200 µg/mL	y=9.8242ln(x)-14.731	0.9888
	FCH	3.1 µg/mL - 200 µg/mL	y=7.0936ln(x)-8.2629	0.9466
	AAH	3.1 µg/mL - 200 µg/mL	y=3.9871ln(x)+14.027	0.9946
	MPH	3.1 µg/mL - 200 µg/mL	y=8.1863ln(x)-0.7793	0.9667
	Neostigmine	1.6 ng/mL - 200 ng/mL	y=18,478ln(x) - 15,074	0.9916

Table S.2.2 – Standard curves used for the quantification of substances equivalent to the standards compounds used in the antioxidant and anticholinesterases assays.

Assay	Reference compound	Amount	Line equation	R^2
DPPH	Trolox	0.25 µg - 4.0 µg	y= -0.3247x + 1.2272	0.9972
DPPH	Quercetin	0.30 µg - 4.8 µg	y= -0.2333x + 1.1766	0.9904
ORAC	Trolox	0.00008 µmol - 0.0025 µmol	y= 33062x + 1.0202	0.9961
AChE	Neostigmine	0.3 ng - 40 ng	y = -0.262ln(x) + 1.0279	0.9916
BChE	Neostigmine	2.5 ng - 160 ng	y = -0.249ln(x) + 1.3293	0.9851

Table S.2.3 – *P* values of the statistical tests referring to the morphological measures and of the exploratory test carried out with the zebrafish larvae submitted to the aqueous extracts of the six studied bamboo species. (*): One-way ANOVA test applied when the analyzed data presented normality.

		Kruskal-Wallis test - *p* value					
Measure	**mg/mL**	**OG**	**PM**	**AA**	**FA**	**FC**	**MP**
Body lenght	0.1	0.0886 *	0.1632	0.1195	0.9024 *	0.1635 *	0.6034
	0.5	0.0055	0.0683	0.0485	0.3258 *	0.0872 *	0.0040
Ocular distance	0.1	0.6134 *	0.6991 *	0.6704 *	0.6485 *	0.8969 *	0.3658
	0.5	0.8771 *	0.8266 *	0.0033 *	0.0017 *	0.0077 *	0.0945
Surface area	0.1	0.6251 *	0.1928 *	0.6100 *	0.0194 *	0.1330 *	>0.9999
	0.5	<0.0001 *	0.0385 *	<0.0001 *	<0.0001 *	0.0109 *	<0.0001
Distance	0.1	0.2106	0.6207 *	>0.9999	>0.9999	0.4070	>0.9999
	0.5	<0.0001	0.2790 *	<0.0001	0.0190	<0.0001	<0.0001
Velocity	0.1	>0.9999	>0.9999	0.9328	0.4790	>0.9999	>0.9999
	0.5	0.0328	0.0546	0.0011	0.1313	>0.9999	0.3579
Moving	0.1	0.2318	0.9745	>0.9999	>0.9999	0.0432 *	>0.9999
	0.5	<0.0001	>0.9999	<0.0001	0.0020	<0.0001 *	<0.0001
Time outside area	0.1	0.8575	0.7591	>0.9999	0.5477	>0.9999	0.2897
	0.5	0.0470	0.5498	0.0464	0.1974	0.2394	>0.9999
Absolute turn angle	0.1	0.1865	0.9243	0.5452	0.9997 *	>0.9999	0.7032
	0.5	<0.0001	0.5351	<0.0001	0.0068 *	<0.0001	0.0251

Table S.2.4 – *P* values of the statistical tests referring to the inhibitory avoidance test carried out with the zebrafish adults submitted to the aqueous extracts of the six studied bamboo species. (T): T test was applied when the analyzed data presented normality.

Mann-Whitney test	***p* values**					
	OGA	**PMA**	**AAA**	**FAA**	**FCA**	**MPA**
	Training vs. Test	**Training vs. Test**	**Training vs. Test**	**Training vs. Test**	**Training vs. Test**	**Training vs. Test**
Saline/H_2O	<0.0001	0.0003	<0.0001	<0.0001 (T)	<0.0001	<0.0001
0.1 mg/kg / H_2O	<0.0001	0.0115	0.0005	0.1252	<0.0001	0.0010
0.5 mg/kg / H_2O	0.0002	0.0070	0.0049	0.4187	0.0061 (T)	0.0011
Saline/Scopolamine	0.8319	0.9881	0.1540	0.9864	0.2170	0.6390
0.1 mg/kg / Scopolamine	0.0279	0.2609	0.0552	0.0573	0.0108	0.0321
0.5 mg/kg / Scopolamine	0.0617	0.6729	0.5182	0.5075	0.0010	0.0067

Table S.2.5 – *P* values of the statistical tests referring to the avoidance behavior test carried out with the zebrafish larvae submitted to the aqueous extracts of the six studied bamboo species.

	One-way ANOVA test - *p* value					
mg/mL	**OGA**	**PMA**	**AAA**	**FAA**	**FCA**	**MPA**
0.1	<0.0001	0.0014	0.1258	0.0008	0.9750	0.3403
0.5	0.0063	0.0141	0.0038	0.0131	0.6050	0.1539

Resumo

Há séculos os povos asiáticos usam as folhas de algumas espécies de bambus para o tratamento de diversas doenças, principalmente doenças de transtorno mental que são atribuídas ao envelhecimento humano. O Brasil possui a maior diversidade de espécies de bambu das Américas, com alto grau de endemismo, mas nada se sabe sobre o potencial medicinal dessas espécies. Portanto, considerando que espécies asiáticas são utilizadas na medicina tradicional e possuem atividades biológicas atribuídas à presença de substâncias fenólicas, a hipótese deste estudo é que os metabólitos secundários, principalmente compostos fenólicos presentes nos bambus brasileiros também possuem ação contra doenças que afetam a cognição e memória. Este trabalho teve como objetivo a caracterização química e a análise do potencial antioxidante e anticolinesterasico dos extratos foliares de *Olyra glaberrima* (OG), *Parodiolyra micranta* (PM), *Aulonemia aristulata* (AA), *Filgueirasia arenicola* (FA), *Filgueirasia canavieira* (FC) e *Merostachys pluriflora* (MP); além de também testar o efeito toxicológico dos extratos aquosos na morfologia, locomoção e comportamento das larvas de zebrafish (*Danio rerio*), e analisar o potencial na melhoria das funções cognitivas e de memória em adultos de zebrafish. As espécies brasileiras de bambu apresentaram grande diversidade de constituintes, foram identificados 181 compostos, entre eles flavonoides, terpenoides, alcaloides, fitoesterois, entre outros. Os extratos hidroetanólico e aquoso de todas as espécies estudadas apresentaram alto potencial antioxidante, especialmente *P. micrantha* e *F. arenicola*. *M. pluriflora* apresentou potencial moderado como inibidor da ação da enzima acetilcolinesterase. Os ensaios *in vivo* mostraram que os extratos aquosos a 0.1 mg/mL não são tóxicos para os embriões de zebrafish, bem como não alteram o comportamento exploratório das larvas. Além disso, os extratos aquosos de OG, PM, AA e FA aumentaram a capacidade cognitiva das larvas de zebrafish frente a um estímulo aversivo. Os extratos aquosos de OG, FC e MP preveniram os déficits de memória em adultos de zebrafish quando tratados com escopolamina. Embora esses metabólitos já tenham sido relatados em outras espécies vegetais, inclusive em espécies asiáticas de bambu, é a primeira vez que são reportados para essas seis espécies brasileiras de bambu. Além disso, muitas substâncias que foram detectadas neste estudo nunca haviam sido descritas em bambus. Este foi o primeiro estudo com espécies herbáceas de bambu; também foi o primeiro estudo que usou zebrafish como modelo experimental para testar extratos de bambu. *M. pluriflora* mostrou-se uma espécie promissora para estudos futuros, devido ao bom desempenho nos bioensaios realizados. No entanto, os estudos sobre o cultivo e a reprodução das espécies brasileiras de bambu ainda são insuficientes, sendo necessários para avaliar se essas espécies são passíveis de cultivo ou se seu manejo sustentável seria viável. Além disso, mais estudos são necessários para entender quais moléculas são responsáveis pelas atividades analisadas, bem como seu mecanismo de ação e biodisponibilidade.

Palavras-chave: compostos fenólicos, antioxidante, anticolinesterase, zebrafish.

Abstract

For centuries, Asian people have used the leaves of some bamboo's species to treat a variety of diseases, mainly mental disorders that are attributed to human aging. Brazil has the greatest diversity of bamboos in the Americas, with a high degree of endemism, but nothing is known bout the medicinal potential of these species. Therefore, considering that Asian species are used in tradicional medicine and have biological activities attributed to the presence of phenolic compounds, the hypotheses of this study is that the secondary metabolites, mainly phenolic compounds from Brazilian bamboos, also have biological potential against diseases that affect cognition and memory. This study aimed to chemical characterize and to analyze the antioxidant and anticholinestare potential of leaf extracts from *Olyra glaberrima* (OG), *Parodiolyra micrantha* (PM), *Aulonemia aristulata* (AA), *Filgueirasia Arenicola* (FA), *Filgueirasia cannavieira* (FC), and *Merostachys pluriflora* (MP); as well as, to analyze the toxicological effect of aqueous extracts on the morphology, locomotion, and behaviour of zebrafish larvae (*Danio rerio*), and to analyze the potential for improving cognitive and memory functions in adults of zebrafish. The Brazilian species of bamboo showed a great diversity of constituents, 181 compounds were identified, amog them flavonoids, terpenoids, alkaloids, phytosterols, among others. Hydroethanol and aqueous extracts of all studied species showed high antioxidant potential, especially *P. micrantha* and *F. arenicola*. *M. pluriflora* showed moderate potential as an inhibitor of the acetylcholinesterase activity. The *in vivo* assays showed that aqueous extracts at 0.1 mg/mL are not toxic for zebrafish embryos, as well as they do not alter the larval exploratory behavior. In addition, the extracts from OG, PM, AA, and FA increased the cognitive capacity of zebrafish larvae against an aversive stimulus. OG, FC and MP aqueous extracts prevented the memory deficit induced by scopolamine treatment. Although these metabolites have already been reported in other plant species, including Asian bamboo species, it is the first time that they have been reported for these six Brazilian bamboo species. Moreover, many substances that were detected in this study had never been described for bamboos. This was the first study with herbaceous bamboo species; it is also the first study that used zebrafish as an experimental model to test bamboo extracts. *M. pluriflora* proved to be a promising species for future studies, due to the good performance in the bioassays performed. However, studies on the cultivation and reproduction of Brazilian bamboo species are still insufficient, being necessary to assess whether these species could be cultivated, or whether their sustainable management would be viable. Besides that, further studies are needed to understand which molecules are responsible the activities analyzed, as well as their mechanism of action and bioavailability.

Key-words: phenolic compounds, antioxidant, anticholinesterase, zebrafish.