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Are Brazilian bamboo species helpful for cognition and memory?

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ABSTRACT

Background: Bamboo species has been used for centuries in Asian traditional medicine to treat mental disorders resulting from the aging process. Brazil has the greatest diversity of bamboos in the Americas, with a high degree of endemism, but just a few of them has been studied for bioprospecting purposes.
Purpose: Considering the previous report on the use of Asian bamboos to treat diseases affecting cognition and memory, a set of Brazilian bamboos species was investigated for similar properties.
Methods: Leaves from *Olyra glaberrima, Parodiolyra micrantha, Aulonemia aristulata, Filgueirasia arenicola, Filgueirasia cannavieira*, and *Merostachys pluriflora* were investigated to their chemical profiles, free radical scavenging, anticholinesterase properties, toxicological effects on zebrafish larvae (*Danio rerio*), and protective effect on memory impairment assays (scopolamine-induced assay).
Results: Based on HPLC-ESI-MS/MS data, 70 compounds had their putative structures assigned based on analysis of molecular formula and fragmentation pattern. The most abundant compounds were glycosylated flavonoids, followed by terpenoids and soluble sugars. Overall bamboo leaf extracts showed high antioxidant potential. A moderate potential as an inhibitor of the acetylcholinesterase activity was detected for *M. pluriflora*. The *in vivo* assays indicated that aqueous extracts at 0.1 mg/ml are not toxic for zebrafish embryos and do not alter the larval exploratory behavior. The extracts from *O. glaberrima, P. micrantha, A. aristulata*, and *F. arenicola* increased the cognitive capacity of zebrafish larvae against an aversive stimulus. In adult zebrafish, the extracts from *O. glaberrima, F. cannavieira*, and *M. pluriflora* prevented the cognitive deficit induced by scopolamine treatment.
Conclusions: Aqueous extracts of the six Brazilian bamboo species showed high *in vitro* antioxidant potential. Some bamboo extracts increased the cognitive capacity of zebrafish larvae in the face of an aversive stimulus. Furthermore, some of them can reduce the memory loss induced by scopolamine in zebrafish adults. 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.

Abbreviation

AA	*Aulonemia aristulata;*
AAPH	2,2′-Azobis (2-amidinopropane) dihydrochloride.
ACh	Acetylcholine;
AChE	Acetylcholinesterase;
ATCI	Acetylthiocholine.
AUC	Area under the curve;
BChE	Butyrylcholinesterase;
BOD	Biochemical Oxygen Demand;
BTCI	Butyrylthiocholine;
CEUA/PUCRS	Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul;
CNS	Central Nervous System;

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DMSO Dimethyl sulfoxide;
dpf days post-fertilization;
DPPH 2,2-diphenyl-1-picrylhydrazyl;
DTNB 5,5′-Dithiobis-(2-nitrobenzoic acid);
EC50 Concentration to reach 50% of the antioxidant activity;
FA *Filgueirasia arenicola;*
FC *Filgueirasia canavieira;*
GNPS Global Natural Products Social Molecular Networking;
hpf hour post-fertilization;
HPLC-ESI-MS/MS High-performance Liquid Chromatography-electrospray Ionization-tandem Mass Spectrometry;
IC50 Concentration to reach 50% inhibition
MP *Merostachys pluriflora;*
OG: *Olyra glaberrima;*
ORAC Oxygen Radical Absorbance Capacity;
PBS Phosphate Buffer Solution;
PM *Parodiolyra micranth;*.
PUCRS Pontifícia Universidade Católica do Rio Grande do Sul

Introduction

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. The most well-known form of dementia is Alzheimer's disease, and the most common signs among all types of dementia are memory loss, and the loss of practical abilities (ADI, 2019).

Cognitive impairment and memory loss are associated with reduced

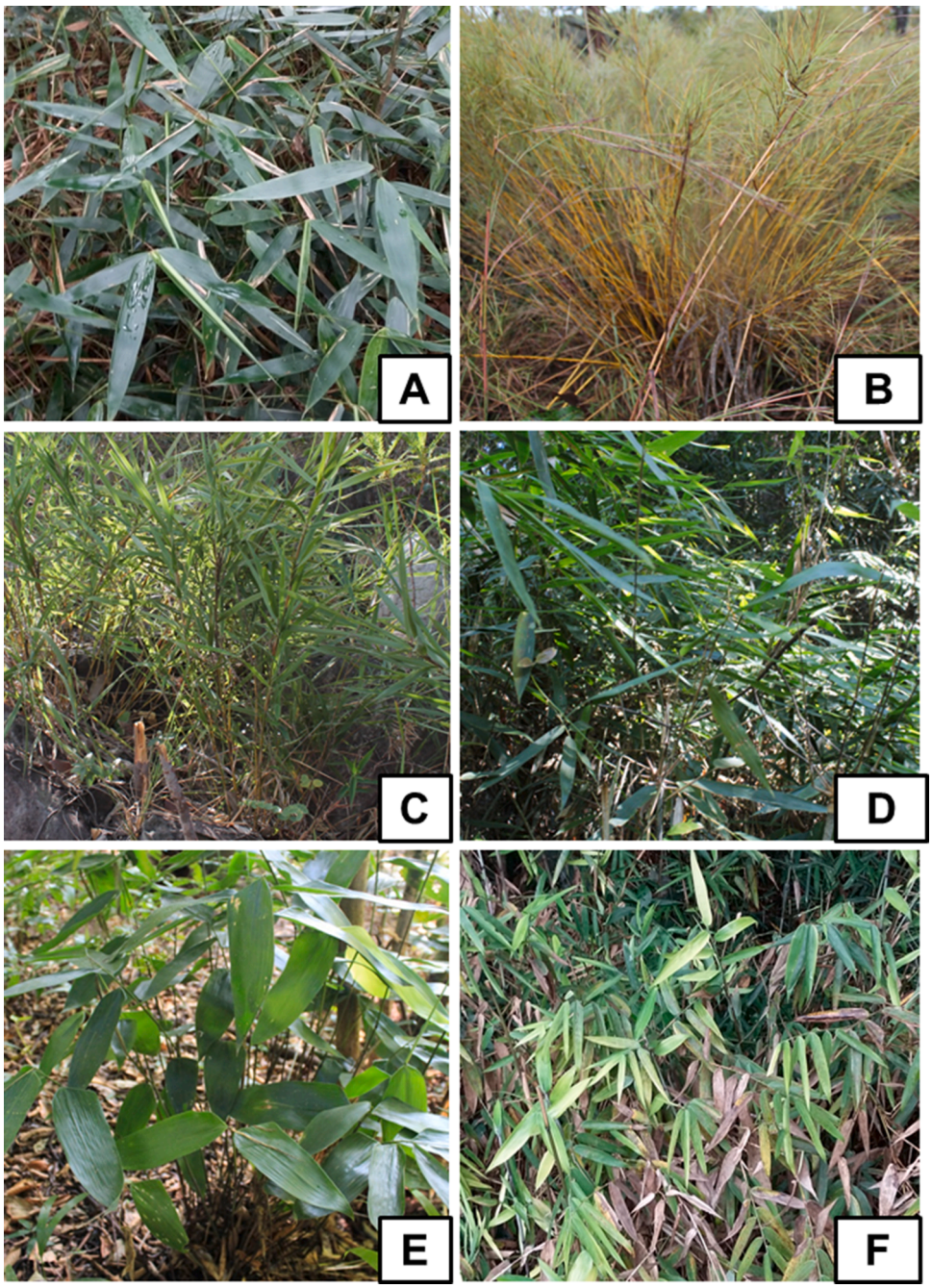

Fig. 1. . Brazilian bamboo species. **(A)** *Aulonemia aristulata*; **(B)** *Filgueirasia arenicola;* **(C)** *Filgueirasia cannavieira;* **(D)** *Merostachys pluriflora;* **(E)** *Olyra glaberrima;* **(F)** *Parodiolyra micrantha.* Photos: (A,D,E,F) – Gagliano J and Furlan CM; (B,C): Grombone-Guaratini MT.

Table 1
Plant collection data.

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

SP: State of São Paulo, Brazil. GO: State of Goias, Brazil. Herbarium SP: Herbarium of the Botanical Institute of São Paulo. Herbarium SPF: Herbarium of the University of São Paulo.

rates of acetylcholine (ACh) in the synaptic process, decreasing cortical cholinergic neurotransmission, and other neurotransmitters Acetylcholine 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).

Natural products have played an important role in ancient traditional medicine systems and are still in common use today. Some phytoconstituents may improve memory as well as cognitive functions, and potentially suppress neurodegeneration of the brain. Many natural products have shown a neuroprotective effect against mental disorders (Essa et al., 2012).

One of the most well-known examples of phytomedicine is *Ginkgo biloba* L. (Ginkgoaceae), used for centuries in traditional Chinese medicine to improve alertness. Currently, ginkgo extracts have been widely used specifically to increase cognitive function, and many of the protective effects of the chronic use of Ginkgo extracts are related to the presence of ginkgolides and flavonoids with antioxidant and anti-inflammatory properties (Gold et al., 2002).

As ginkgo, extracts from Asian bamboo species have also traditional use for the treatment of mental illness. For example, an ethnobotanical survey in a rural district of Benin, West Africa, showed the use of *Oxytenanthera abyssinica* (A. Rich.) Munro, *Bambusa vulgaris* Schrad. ex J. C. Wendl., and *Dendrocalamus asper* (Schult. & Schult. f.) Backer ex K. Heyne (Poaceae) leaves for treating nervous system disorder, as well as for memory issues by 15% of the informants (Honfo et al., 2015).

High conservation between zebrafish (*Danio rerio*) 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). Zebrafish has their genome well-characterized and conserved, with over 70% of their 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 (Fontana et al., 2018).

Considering the pharmacological and physiological similarities with mammals, zebrafish provides a useful and complementary biological model for the discovery of natural drug-like products (Pitchai et al., 2019). Zebrafish, in every developmental stage, can be used as animal models for toxicological studies. Embryos and larvae are useful for toxicological assessments due to the transparent nature of the egg, which allows the direct observation of developmental stages and the requirement of small quantities of test compounds. Adult zebrafish presents a wide behavioral repertoire, exhibiting a wide range of complex behaviors, including social, motor, affective and defensive, that may be useful for modeling various CNS disorders (Fontana et al., 2018).

Considering that Asian species of bamboos have been used in traditional medicine for the treatment of mental disorders, the present study hypothesized that secondary metabolites presented in Brazilian bamboo extracts would also have biological potential against diseases that affect cognition and memory.

Therefore, this study aimed to perform the chemical characterization of the aqueous extracts of six Brazilian bamboo species, as well as to evaluate the toxicity of these extracts evaluating the morphology and behavior of zebrafish larvae, and their potential for improving cognitive and memory functions in adults of zebrafish and their antioxidant and anticholinesterase potential.

Material and methods

Plant species

Six Brazilian bamboo species were studied, four of them belonging to the Bambuseae (tropical woody bamboo): *Aulonemia aristulata* (Döll) McClure (AA); *Filgueirasia arenicola* (McClure) Guala (FA); *Filgueirasia cannavieira* (Alvaro da Silveira) Guala (FC); and *Merostachys pluriflora* Munro ex. E.G.Camus (MP); and two species from Olyreae (herbaceous bamboo): *Olyra glaberrima* Raddi (OG) and *Parodiolyra micrantha* (Kunth) Davidse & Zuloaga (PM) (Poaceae) (CNPq Permit # 010,006/2015–0). All plant names were checked with http://www.theplantlist.org (accessed in October 2021) (Fig. 1, Table 1).

Plant extraction

Dried (40 °C) and powdered leaves were immediately immersed in 500 ml of sterile, freshly boiled distilled water for 20 min. Aqueous extracts were evaporated under reduced pressure below 50 °C and freeze-dried. Leaf samples were composed by a pool of individuals characterizing a single composite sample for each species.

HPLC-ESI-MS/MS

All extracts were solubilized in 80% methanol (5 mg/ml), filtered (PTFE 0.45 μm) and submitted by HPLC-ESI-MS/MS using an LC Shimadzu (Kyoto, Japan) equipped with a controller (CBM-20A), two pumps (model LC-20AD), an automatic injector (SIL-20AHT), a column oven (CTO-20A), and a UV/Vis detector (SPD-20A). Chromatographic separation used a Kinetex C-18 column (Phenomenex, 100 A, 100 × 1 mm, 2.6 μm PFP) at 40 °C, 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 acetonitrile (0.1% acid formic, acidified-ACN), increasing acidified-ACN 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 (Bruker MicrOTOF-QII) operated in positive and negative mode, nitrogen was used as a nebulizer (4 Bar) and dried gas (flow of 8 l/min). The capillary voltage was set at 4500 V and drying temperature to 200 °C. 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.

All raw data files obtained from the HPLC analysis of the extracts were converted to .mzXML using Data Analysis 4.3 software (Bruker) 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.

GNPS provides the ability to analyze a data set and to compare it to all available data which combines a catalog of over 221,000 MS/MS reference library spectra from 18,163 compounds. 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). In this work, only substances that had a cosine index equal to and/or greater than 0.70 were integrated.

The quantification of the identified compounds was made using the area (MaU) of each constituent using the following formula: (% relative) = [(IMaU*100)/TMaU]; where (IMaU) is the individual area obtained, and the (TMaU) is the sum of all areas of the compounds identified.

In vitro biological assays

Antioxidant and anticholinesterase analyzes were performed in methodological triplicate, and the measures were done using Synergy H1 equipment (BioTek, Inc.). The IC_{50} and EC_{50} were calculated by using a regression equation between the extract concentration and the percentage of enzyme inhibition and antioxidant activity of each sample, respectively. All results were expressed in μg/ml.

AChE and BChE assays

AChE (type VI-S lyophilized powder, 987 U/mg solid) and BChE (lyophilized powder, 140 U/mg solid) both from electric eel, were used for preparing stock solutions (AChE 493.5 U/ml and BChE 140 U/ml) kept at −80 °C. 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 $MgCl_2$. ATCI and BTCI, both at 18 mM, were dissolved in ultrapure water. In to a 96-well plate 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 (0.03 – 2 mg/ml) in Tris–HCl buffer. After mixing, the plate was incubated for 15 min (25 °C). The enzymatic reaction was initiated by the addition of 20 μl of 18 mM ATCI or BTCI and the hydrolysis was monitored at 412 nm by reading the absorbance every 5 min for 20 min (Mathew and Subramanian, 2014). Neostigmine (1.6 ng/ml – 200 ng/ml) was used as a positive control and Tris–HCl buffer as negative control.

DPPH assay

DPPH solution in methanol (0.20 mM) was freshly prepared and 200 μl was mixed with 20 μl of each sample (0.00125 – 1 mg/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 (Santos et al., 2016). Trolox (12.5 – 200 μg/ml) was used as a positive control, while methanol and 10% DMSO as a negative control.

ORAC assay

Sodium fluorescein was dissolved in 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 mixed with each sample (in PBS) at different concentrations (0.97 – 125 μg/ml). The reaction mixtures were incubated for 30 min at 37 °C and 75 mM AAPH solution was added. As a positive control, a PBS Trolox solution was plotted (6.25 – 50 mM) and PBS as a negative control. 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 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 (Santos et al., 2016). The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net AUC.

In vivo biological assays

For all *in vivo* assays, the aqueous extracts at four different concentrations were used (1.5, 1.0, 0.5, and 0.1 mg/ml in water). All protocols were approved by the Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul (9413, CEUA/PUCRS).

Embryos and larvae 0–5 days post-fertilization (dpf) and adult animals (12–18 months, 0.3–0.6 g) of wild-type *D. rerio*, from the AB background, were obtained from PUC-RS breeding colony. A total of 2400 embryos and 360 adult animals were used for the development of this study. The animals were maintained in recirculating systems (Zebtec, Tecniplast, VA, 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 a light/dark cycle of 14/10 h, 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).

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 and immediately subjected to the treatment. The embryos were maintained for up to 5 dpf at a density of one larva *per* 2 ml in Petri dishes in a 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.

Analysis in zebrafish embryos and larvae

Survival rate

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 hpf to 5 dpf). Animals were monitored daily for survival rate ($n = 80$, *per* group) using an inverted stereomicroscope (Nikon, Melville, NY, USA). After this treatment, the animals were subjected to experimental tests. The hatching rate was also monitored; however, there was no difference between the groups (data not shown).

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). 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).

Table 2
Constituents from aqueous extracts analyzed by HPLC-ESI-MS/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 (%) OG	PM	AA	FA	FC	MP	Cosine	Compound classes
1	6.56	Hyperoside	0.0	0.0	0.0	1.0	0.0	0.0	0.80	Flavonol
2	7.82	Cordycepin	1.0	0.0	1.0	0.0	0.0	0.0	0.95	Nucleoside derivative
3	8.02	Inosine	0.0	0.4	0.0	0.9	0.0	0.0	0.93	Nucleoside
4	14.52	Succinoadenosine	1.8	0.0	0.2	0.7	0.8	0.3	0.88	Nucleoside derivative
5	15.95	Tryptophan *N*-glucoside	13.4	6.2	2.0	0.0	0.0	0.0	0.82	Amino acid
6	16.25	(3*R*,5*S*)−4-[(*E*)−3-(3,4-D*ihydroxyphenyl*)*prop*-2-*enoyl*]oxy-1,3,5-trihydroxycyclohexane-1-carboxylic acid	0.0	0.2	0.1	0.0	0.0	0.0	0.73	Hydroxycinnamic acid derivative
7	16.35	Chlorogenic acid	0.0	0.7	1.1	0.0	0.0	0.0	0.77	Hydroxycinnamic acid derivative
8	16.87	L-Tryptophan	0.0	0.0	0.2	0.0	0.0	0.0	0.77	Amino acid
9	18.26	Kaempferol 3-glucuronide	0.0	0.0	0.9	0.0	0.0	0.8	0.85	Flavonol
10	19.62	Licoagroside B	0.0	0.0	1.2	0.0	0.0	0.0	0.89	Soluble sugar derivative
11	20.94	Riboflavin	1.8	0.3	4.3	0.6	0.9	1.7	0.86	Vitamin
12	22.14	Apigenin 6,8-digalactoside	1.4	0.4	0.2	0.0	0.0	0.1	0.77	Flavone
13	23.71	Arillatose B	0.0	0.4	0.0	0.0	0.0	0.0	0.85	Soluble sugar derivative
14	23.96	Vicenin	8.8	17.4	24.6	0.8	8.1	45.4	0.94	Flavone
15	25.70	Sibiricose A1	0.0	0.2	0.0	0.0	0.0	0.0	0.75	Hydroxycinnamic acid derivative
16	25.71	Corymboside	1.3	0.0	1.9	0.5	0.0	0.1	0.93	Flavone
17	26.47	(*E*)-*N*-(4-Acetamidobutyl)−3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide	0.0	0.0	7.1	0.0	0.0	1.1	0.73	Phenylamide
18	27.39	5,7-Dihydroxy-2-phenyl-6,8-bis[3,4,5-*trihydroxy*-6-(*hydroxymethyl*)*oxan*-2-*yl*]chromen-4-one	0.0	9.1	0.0	27.8	18.7	15.0	0.72	Flavonol
19	28.11	Saponarin	8.8	3.4	0.0	3.9	0.0	0.0	0.86	Flavone
20	29.14	Schaftoside	2.6	0.2	1.2	4.8	1.2	8.5	0.93	Flavone
21	29.75	Vitexin −4′′-*O*-glucoside	0.0	0.0	0.0	0.0	0.0	0.6	0.80	Flavone
22	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	1.7	5.0	0.0	0.0	0.0	0.0	0.97	Flavone
23	30.24	2′′-*O*- β -D-Xylopyranosylorientin	0.0	0.0	3.8	0.0	0.0	0.0	0.88	Flavone
24	30.91	Swertisin	0.0	0.2	0.0	0.0	0.0	0.0	0.73	Flavone
25	30.92	Isoorientin	0.0	2.7	0.1	5.9	6.3	0.9	0.91	Flavone
26	31.42	Tricin 5-glucoside	0.0	13.5	2.6	22.4	8.6	3.7	0.96	Flavone
27	32.41	Isovitexin	2.6	0.1	0.9	0.0	10.3	3.1	0.89	Flavone
28	32.69	Quercetin 3,4′-*O*-diglucoside	0.0	0.0	0.9	0.0	0.0	0.0	0.89	Flavonol
29	33.03	Isovitexin 2′-*O*-arabinoside	0.0	0.0	15.8	0.0	0.0	1.1	0.93	Flavone
30	33.61	Syringetin-3-*O*-glucoside	2.7	3.2	0.0	0.0	0.0	1.2	0.79	Flavonol
31	33.73	C-Hexosyl-chrysoeriol *O*-hexoside	1.9	0.0	0.0	0.0	0.0	2.0	0.83	Flavone
32	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	4.4	0.0	19.8	0.0	0.93	Flavonol
33	33.87	Vitexin	0.0	0.0	0.0	16.9	0.0	0.3	0.82	Flavone
34	33.96	Afzelin	0.0	0.3	3.1	0.0	0.0	0.0	0.82	Flavone
35	34.02	6-*O*-Caffeoylarbutin	0.0	0.3	0.0	0.0	0.0	0.0	0.71	Hydroxycinnamic acid derivative
36	34.48	5,7-Dihydroxy-2-(4-hydroxyphenyl)−6,8-bis(3,4,5-trihydroxyoxan-2-yl)chromen-4-one	6.8	23.5	0.0	0.0	0.0	0.0	0.80	Flavone
37	36.07	(2*Z*)−4,6-Dihydroxy-2-[(4-*hydroxy*-3,5-*dimethoxyphenyl*)*methylidene*]−1-benzofuran-3-one	0.0	0.0	0.2	0.1	0.0	0.1	0.72	Aurone
38	37.73	Isoscoparin	0.0	0.0	0.0	0.7	0.0	0.0	0.79	Flavone
39	40.52	*C*-Hexosyl-chrysoeriol	0.0	0.3	0.0	0.0	0.0	0.0	0.73	Flavone
40	40.52	Peonidin 3-galactoside	0.0	0.0	1.4	0.0	0.0	0.0	0.90	Anthocyanin
41	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	0.8	0.0	0.0	0.0	0.0	0.93	Flavone
42	45.55	3-(alpha-L-Rhamnopyranosyloxy)−8-(beta-D-glucopyranosyloxy)−3′,4′,5-trihydroxy-7-methoxyflavone	0.6	0.0	0.0	0.0	3.6	0.0	0.78	Flavone
43	45.73	Chrysoeriol 7-*O*-Neohesperidoside	0.0	0.3	0.0	0.0	0.0	0.0	0.74	Flavone
44	45.73	Apigetrin	0.0	0.4	0.0	0.0	0.0	0.0	0.90	Flavone
45	45.92	Apigenin 4′-*O*-glucoside	0.0	1.5	0.0	0.0	0.0	0.0	0.97	Flavone
46	46.18	Matairesinol	0.0	0.0	0.0	0.0	10.0	0.0	0.92	Lignan
47	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	0.2	0.0	0.0	0.0	0.0	0.0	0.90	Flavonol
48	46.40	3-[(2*S*,3*R*,4*S*,5*S*,6*R*)−4,5-Dihydroxy-6-(hydroxymethyl)−3-[(2*S*,3*R*,4*S*,5*R*,6*R*)−3,4,5-*trihydroxy*-6-(*hydroxymethyl*)*oxan*-2-*yl*]oxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxyphenyl)−7-methoxychromen-4-one	4.1	0.0	0.0	0.0	5.3	0.0	0.90	Flavonol
49	47.63	Tricin 7-glucuronide	0.0	0.0	0.0	0.3	0.4	0.0	0.83	Flavone
50	54.23	Spinacetin 3-gentiobioside	5.6	1.2	0.0	0.0	0.0	2.6	0.87	Flavonol
51	56.13	Costunolide	0.0	0.0	0.0	1.8	0.0	0.0	0.70	Sesquiterpene lactone
52	60.04	3-*p*-Coumaroylquinic acid	0.0	0.0	0.0	0.0	0.0	2.8	0.74	

(*continued on next page*)

Table 2 (*continued*)

Peak	R.T. (min)	Constituent	Relative percentage (%) OG	PM	AA	FA	FC	MP	Cosine	Compound classes
										Hydroxycinnamic acid derivative
53	63.08	Apaensin	0.0	0.8	0.0	0.0	0.0	0.0	0.70	Chromene
54	76.30	2′,5,6-Trimethoxyflavone	2.6	0.1	0.2	0.3	0.2	1.2	0.89	Flavone
55	78.59	Docosanol	0.5	0.5	0.4	0.8	0.0	0.1	0.97	Aliphatic alcohol
56	80.12	(8,8-Dimethyl-2,10-dioxo-9*H*-pyrano[2,3-*f*]chromen-9-yl) (*Z*)−2-methylbut-2-enoate	3.0	0.7	0.3	0.1	0.0	0.3	0.94	Chromen
57	81.66	Juniperoside III	0.0	1.3	0.0	0.0	0.0	0.0	0.71	Phenylglycoside
58	83.53	Enniatin B	0.0	0.0	0.0	0.5	0.0	0.0	0.83	Cyclic depsihexapeptides
59	83.95	1-Hexadecanoyl-2-(9*Z*,12*Z*-octadecadienoyl)-sn-glycero-3-phospho-(1′-sn-glycerol)	0.0	0.3	0.0	0.0	0.0	0.0	0.77	Glycerophospholipids
60	84.00	1-Stearoyl-2-linoleoylphosphatidylcholine	0.0	0.0	0.0	0.9	0.0	0.0	0.74	Glycerophospholipids
61	84.44	Campesterol	0.0	0.0	0.0	0.0	1.0	0.0	0.80	Phytosterol
62	85.03	β-Sitosterol	0.0	0.0	0.1	0.6	0.0	0.9	0.75	Phytosterol
63	85.99	Avobenzone	0.9	0.5	3.8	0.0	0.8	0.9	0.94	Dihydrochalcones
64	87.25	1-Palmitoyl-2-homo-g-linolenoyl-sn-glycero-3-phosphocholine	0.0	0.0	1.8	0.0	0.0	0.0	0.93	Glycerophospholipids
65	87.65	*N*-(11*Z*-eicosenoyl)−1-β-galactosyl-4E,14Z-sphingadienine	1.2	1.6	1.9	0.0	1.8	1.6	0.88	Sphingolipids
66	88.07	Eudesmin	0.0	0.0	0.0	0.7	0.0	0.0	0.85	Lignan
67	91.05	Koaburside	0.0	0.0	1.8	0.0	0.0	1.8	0.71	Phenylglycoside
68	91.25	α-Tocopheryl acetate	0.0	0.0	0.0	2.0	0.0	0.0	0.88	Vitamin
69	91.27	Sarmentoside B	14.0	0.2	10.5	5.2	2.1	1.6	0.76	Cardenolide
70	92.06	Bidwillon A	10.9	1.9	0.0	0.0	0.0	0.0	0.71	Isoflavone

Behavioral analysis in zebrafish larvae

Exploratory behavior

The exploratory behavior of the 5 dpf ($n = 18–36$, *per* group) larvae exposed to bamboo extracts was analyzed. 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. 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 behavior of the animals was video recorded during 5 min. after the 60 s.-habituation period for automated analysis by Ethovision XT 10.0 software (version 11.5, Noldus; 30 frames *per* second video-sampling rate) (Creton, 2009). Distance (cm), time mobile (s), velocity (cm/s) (ratio between distance traveled and time mobile) were quantified and considered as measures of exploration of a new environment and swim activity. It was also quantified absolute turn angle (°), which represents the change in direction of the center point of the animal between two consecutive samples irrespective of the direction of turn and evaluates erratic movements. Last, it was quantified the time spent (s) at the outer ring area of the well, thigmotaxis, as an indicator of anxiety-like behavior.

Avoidance behavior

After the exploratory evaluation of larvae (5 dpf) from bamboo extracts exposure, the animals were placed in a 6-well plate (five larvae *per* well, $n = 20–40$, *per* group) over an LCD monitor, which was presented a video with a visual stimulus (a 1.35 cm diameter red bouncing ball) for measuring cognitive ability and avoidance responses (Pelkowski et al., 2011), during a 5 min-session following 2 min. of acclimation. 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 animals used for avoidance behavior evaluation were selected from different plates, using the same animals for survival analysis. The number of larvae on the non-stimuli area during 5 min. the session was considered indicative of their cognitive ability.

Analysis in adult zebrafish

Inhibitory avoidance task

Adult zebrafish ($n = 360$) were anesthetized by immersion in 0.1 g/l tricaine solution (ethyl-3-aminobenzoate methane sulfonate 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″) × 31 G Short Needle (Becton Dickinson and Company, Franklin Lakes, NJ, USA) (Kinkel et al., 2010). Drug doses and administration routes were chosen and adjusted based on toxicity presented in larval stage animals. After treatment, the 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 a control group) were injected 2 h before the beginning of each experiment. One h before the start of the behavioral assay, the animals were transferred to a tank containing 200 μM scopolamine solution ((−)-Scopolamine hydrobromide trihydrate), dissolved in the system water. Animals that did not receive scopolamine were also transferred to another tank with water to control for handling effects (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. to 12 p.m. Training and test sessions were performed with a 24 h interval between them. In each session, the 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 min. 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 two electrodes attached to an 8.8 V stimulator were used for a final shock pulse of 3 ± 0.2 V AC (intensity measured between electrodes and the center of the dark compartment) for three seconds. 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 time ceiling were imposed

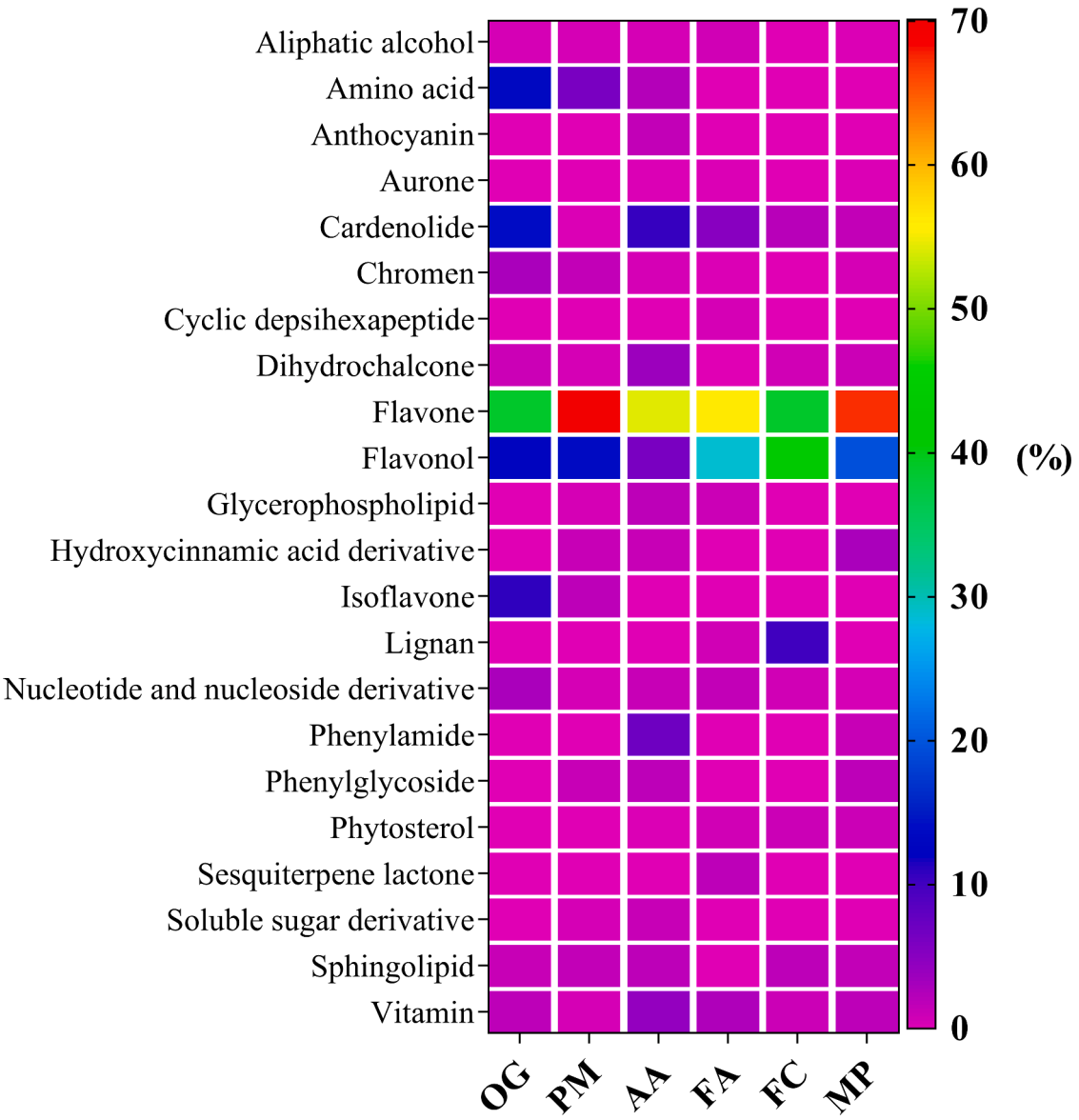

Fig. 2. . Heat map of the relative percentage of the constituents present in aqueous extracts analyzed by HPLC-ESI-MS/MS in six Brazilian bamboo species. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata*; **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora*.

on training and test session latency measurements, respectively.

Statistical analysis

Survival rates 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 a two-way ANOVA test. Latencies of multiple groups were compared by Mann-Whitney U or Student's *t*-tests depending on the normality of the data (assessed by the Shapiro-Wilk test). For all comparisons, the level of significance was defined as $p \leq 0.05$. GraphPad Prism version 8.0.2 program was used for statistical analysis, to build the heat maps and graphics.

Results and discussion

A variety of compounds belonging to different classes of metabolites have been detected. Among them, flavonoids, chalcones, chromenes, aurones, lignans, anthocyanins, and hydroxycinnamic acid derivatives, cardenolides, phytosterols, and sesquiterpene lactones. Some nitrogen compounds were also detected, as well as glycerophospholipids, sphingolipids, soluble sugar derivatives, and vitamins (Table 2, Tables A.1 and A.2).

Despite a large variety of compounds from different classes observed, flavonoids especially flavones, were ubiquitous. For instance, the flavones vicenin, schaftoside, and 2′,5,6-trimethoxyflavone were observed in all samples. However, some of them were more restricted as observed in Table 2.

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* (Lodd. ex Lindl.) Munro, *Sasa kurilensi* (Rupr.) Makino & Shibata, and *Bambusa nutans* Wall. ex Munro (Poaceae) (Van Hoyweghen et al., 2014; Yang et al., 2017; Pande et al., 2018). Even though quercetin was not frequently found in bamboo species, it was previously reported for *A. aristulata* (Grombone-Guaratini et al., 2009), and in the present study, quercetin 3,4′-*O*-diglucoside was detected.

Table 3
In vitro antioxidant and acetylcholinesterase activities of aqueous extracts from bamboo leaves. **(OG)** *Olyra glaberrima*; **(PM)** *Parodiolyra micrantha*; **(AA)** *Aulonemia aristulata*; **(FA)** *Filgueirasia arenicola*; **(FC)** *Filgueirasia cannavieira*; **(MP)** *Merostachys pluriflora*.

Samples	DPPH EC_{50} µg/ml	ORAC EC_{50} µg/ml	AChE IC_{50} µg/ml
OG	325.23	107.37	696.63
PM	118.85	36.50	425.20
AA	215.38	70.77	2091.57
FA	150.20	38.77	873.26
FC	171.03	45.36	609.44
MP	202.01	78.87	423.24
Trolox	94.68	0.24	*
Neostigmine	*	*	0.02

*: not applied. EC_{50}: Concentration to reach 50% of the antioxidant activity. IC_{50}: Concentration to reach 50% inhibition.

The flavone class was the most expressive in the six species, and interestingly, all species also showed high levels of flavonols, which has not been frequently reported for bamboos (Fig. 2).

All samples presented high *in vitro* antioxidant potential (Table 3, Table B.1). For instance, in the ORAC assay, most of these extracts showed EC_{50} below 100 µg/ml (36.50 µg/ml to 107.37 µg/ml), among which the most promising samples were PM and FA (36.50 µg/ml and 38.77 µg/ml, respectively). The EC_{50} for DPPH assay ranged from 118.85 µg/ml in PM to 325.23 µg/ml in OG, the PM was the most active sample supporting the antioxidant properties detected in the ORAC assay.

For anticholinesterase assays, all samples were able to inhibit the action of AChE enzyme with the lowest IC_{50} observed for MP (423.24 µg/ml) and PM (425.20 µg/ml) (Table 3, Table B.1). None of the samples inhibited the BChE activity.

The free radical scavenging activity of the bamboo extracts varied according to the assays. Although both methods have widely been used, they have methodological limitations (Karadag et al., 2009). However, complex samples such as crude extracts reaching EC_{50} below 100 µg/ml are considered promising for further *in vivo* assays (Cos et al., 2006).

The antioxidant capacity of bamboo extracts is widely studied mainly in Asian species. In the last decade, half of the studies on the biological activities of bamboo evaluated the antioxidant potential of these species (Gagliano et al., 2021). Furthermore, the leaf extracts of some *Phyllostachys* species are used as food antioxidant additives 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).

Although there are reports in the literature of the traditional use of bamboo leaf extracts for the treatment of mental disorders and to improve memory, few studies have been conducted so far to investigate the potential action of bamboo extracts on cognition and memory.

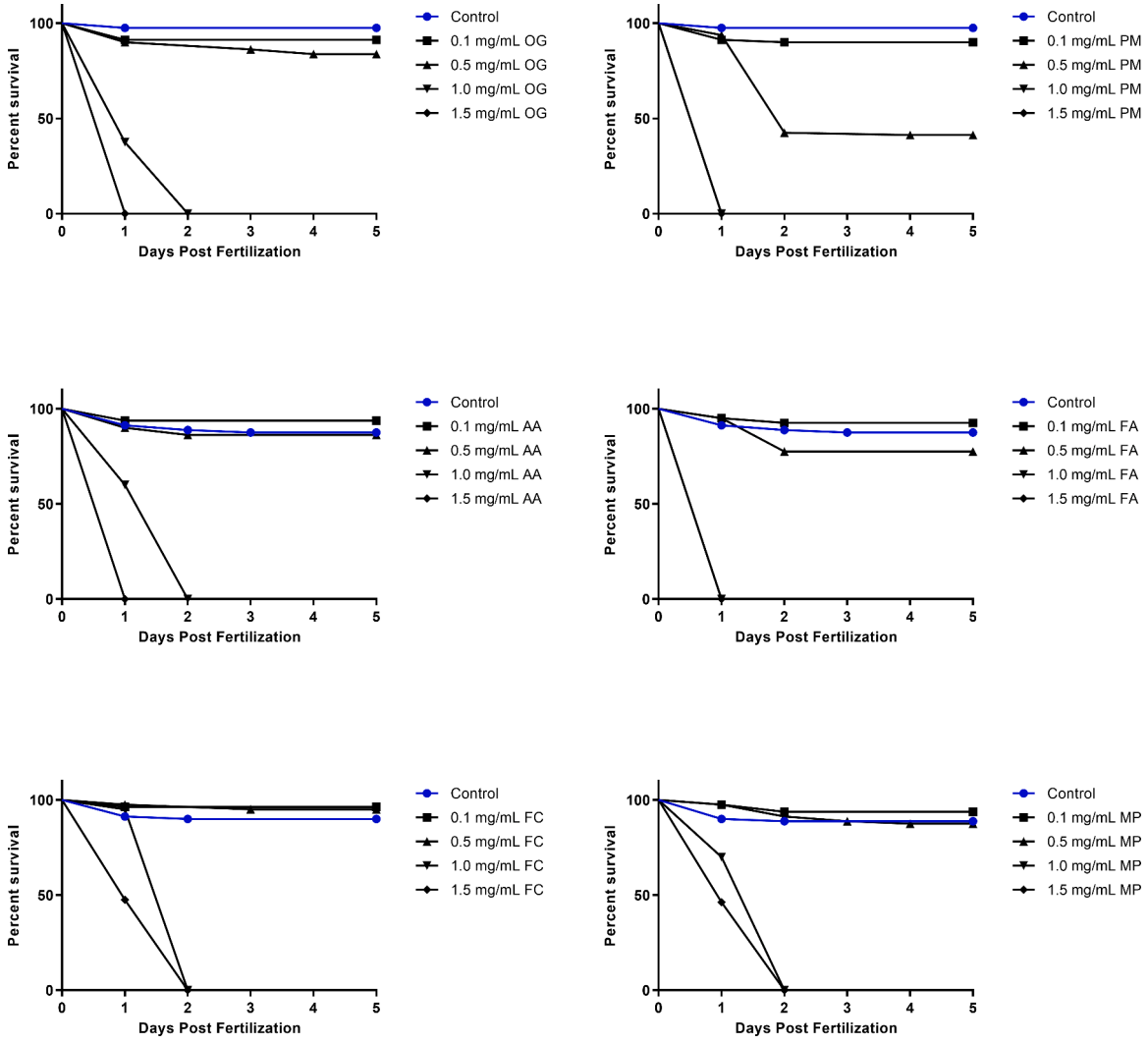

Fig. 3. . Survival rate (%) of zebrafish embryos treated with different concentrations of Brazilian bamboo aqueous extracts. $n = 2.400$ embryos.

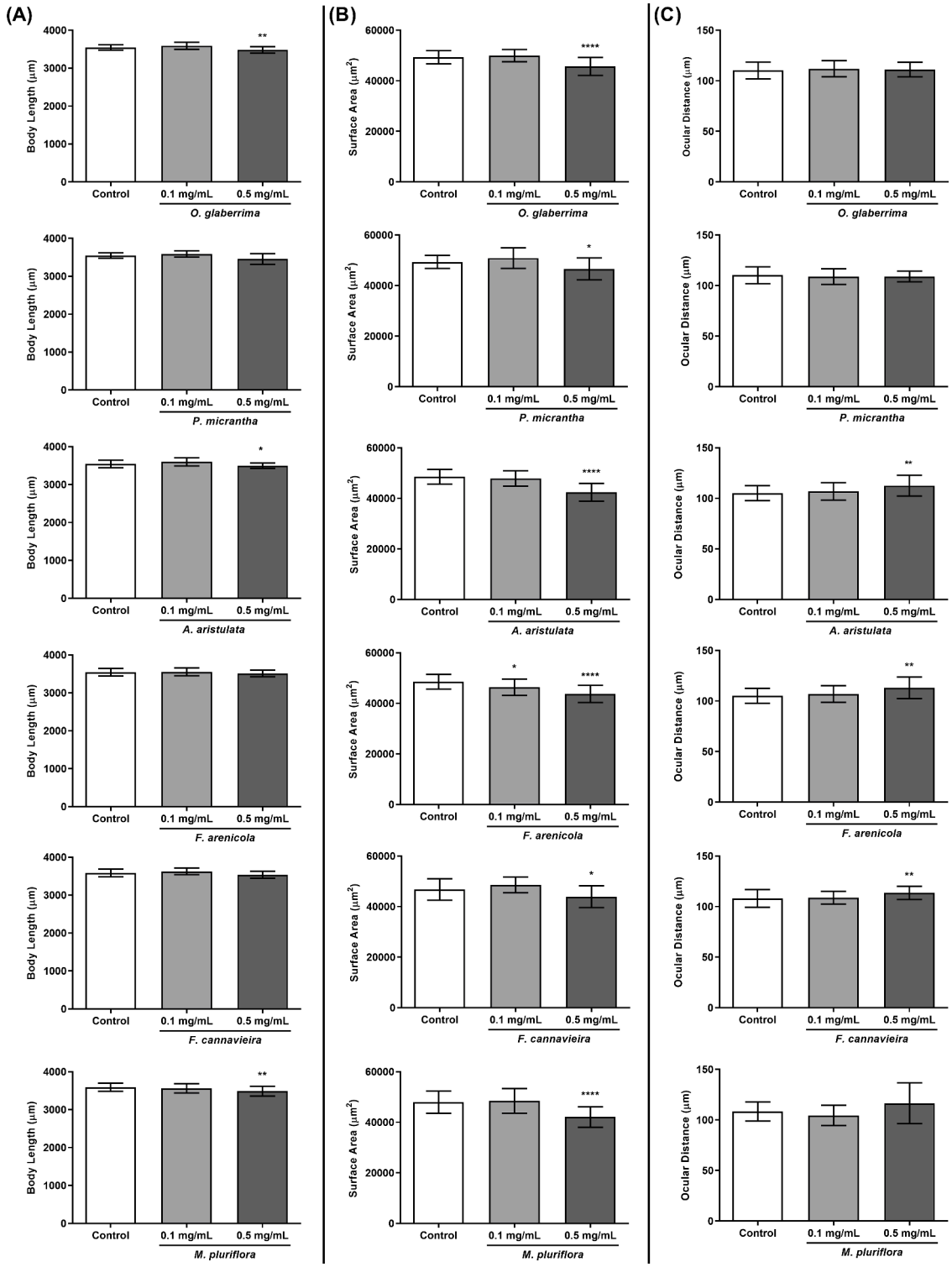

Fig. 4. . Morphological measures: **(A)** Body length (µm), **(B)** Surface area of the eyes (µm^2), **(C)** Ocular distance (µm) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, AA, FA, FC, MP (0.1 and 0.5 mg/ml): $n = 30$ / PM (0.1 mg/ml): $n = 30$; and (0.5 mg/ml): $n = 15$.

The bamboo leaf extract was reported to improve the spatial learning ability of dementia in rats, and that the hippocampus of these model rats showed reduced levels of acetylcholine, epinephrine, norepinephrine, and dopamine, but increased activities of acetylcholine esterase and monoamine oxidase (Liu et al., 2015). Animals treated with bamboo extract had significant inhibition of the enzyme activity compared with untreated rats, suggesting that bamboo extracts might be a potential drug in treating impairment of spatial memory in 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 (Poaceae), a native bamboo from Northeast India, Bangladesh, Nepal, and Myanmar. The major constituent from fruits was identified as verbacine, which showed IC_{50} of 16.01 µg/ml in AChE inhibition assay,

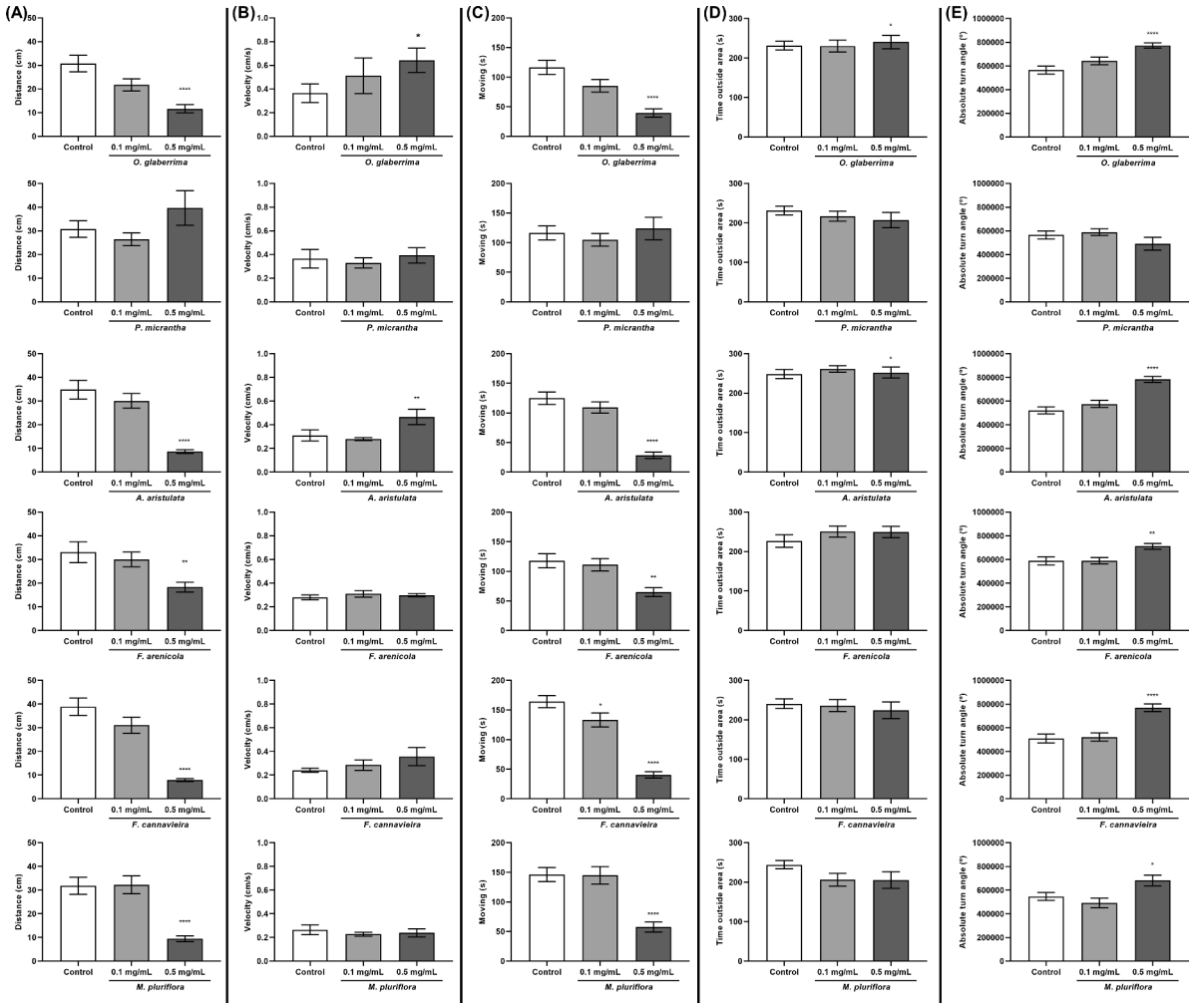

Fig. 5. . Exploratory behavior: **(A)** Distance traveled (cm), **(B)** velocity (cm/s), **(C)** time moving (s), **(D)** time outside area (s), **(E)** absolute turn angle (°) of the zebrafish larvae treated with different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. 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): $n = 18$.

similar to observed for the standard Donepezil (12.91 μg/ml) (Govindan et al., 2018).

Flavonoids have also shown to be promising molecules to inhibit cholinesterase activity. Choi et al. (2014) observed that apigenin and its *C*-glycosylated derivatives showed anticholinesterase potential (both AChE and BChE), and the *C*-glycosylated derivatives vitexin and isovitexin had lower IC_{50} when compared with apigenin.

All bamboo samples showed a diversity of *C*-glycosylated flavones (Table 2), but MP showed the highest abundance of some flavones as vicenin (45.38%). Furthermore, vitexin −4′′-*O*-glucoside and 3-*p*-coumaroylquinic acid were detected only in MP and therefore, these compounds may have contributed to the better anticholinesterase potential of MP when compared to the other species.

All bamboo samples, in their highest concentration (1.0 and 1.5 mg/ml), showed a lethal effect on zebrafish larvae; none of the animals treated with these concentrations survived (Fig. 3).

Larvae exposed to OG ($p = 0.0055$), AA ($p = 0.0485$), and MP ($p = 0.0040$) at 0.5 mg/ml showed a smaller body size when compared to larvae on the control group (Fig. 4A). However, larvae exposed to 0.5 mg/ml of all samples showed significant smaller surface area of the eyes (Fig. 4B), and zebrafish larvae exposed to AA ($p = 0.0033$), FA ($p = 0.0017$), and FC ($p = 0.0077$) at 0.5 mg/ml increased their ocular distance (Fig. 4C), when compared to the control group (Table C.1).

Treatments with the 0.5 mg/ml of the bamboo extracts were not lethal but caused significant morphological changes in the zebrafish larvae. Thus, it was possible to infer that concentrations lower to 0.5 mg/ml were the safest for a subchronic exposure experiment.

Zebrafish larvae were individually submitted for a session of exploratory behavior analysis (Table C.1). Larvae treated with OG, AA, FC, and MP at 0.5 mg/ml ($p < 0.0001$), as well as FA ($p = 0.0190$), traveled shorter distances when compared to the control group (Fig. 5A). However, larvae treated with 0.5 mg/ml of the AA ($p = 0.0011$) and OG ($p = 0.0328$) extracts showed an increase in the swimming speed (Fig. 5B). It was also possible to observe that animals treated with OG, AA, FC, MP ($p < 0.0001$), and FA ($p = 0.0020$) at 0.5 mg/ml; and FC at 0.1 mg/ml ($p = 0.0432$) moved much less when compared to the animals in the control group (Fig. 5C).

The time spent, and location in each plate-well position (outside *vs.* inside area) were considered an index of anxiety, as the zebrafish tends to prefer the corners of the plate (Siebel et al., 2015). For time spent in each plate-well, larvae treated with AA ($p = 0.0464$) and OG ($p = 0.0470$) at 0.5 mg/ml showed significant differences in their behavior when compared to the control group (Fig. 5D). The larvae treated with 0.5 mg/ml of OG, AA, FC ($p < 0.0001$), FA ($p = 0.0068$), and MP ($p = 0.0251$) extracts presented higher absolute turn angle than the control group (Fig. 5E). No significant differences were observed for the location of the larvae in the plate and, therefore, the extracts did not change animals' anxiety behavior.

Immediately after hatching, the zebrafish larvae exhibit vigorous locomotor activity and predatory behavior. This simple predatory

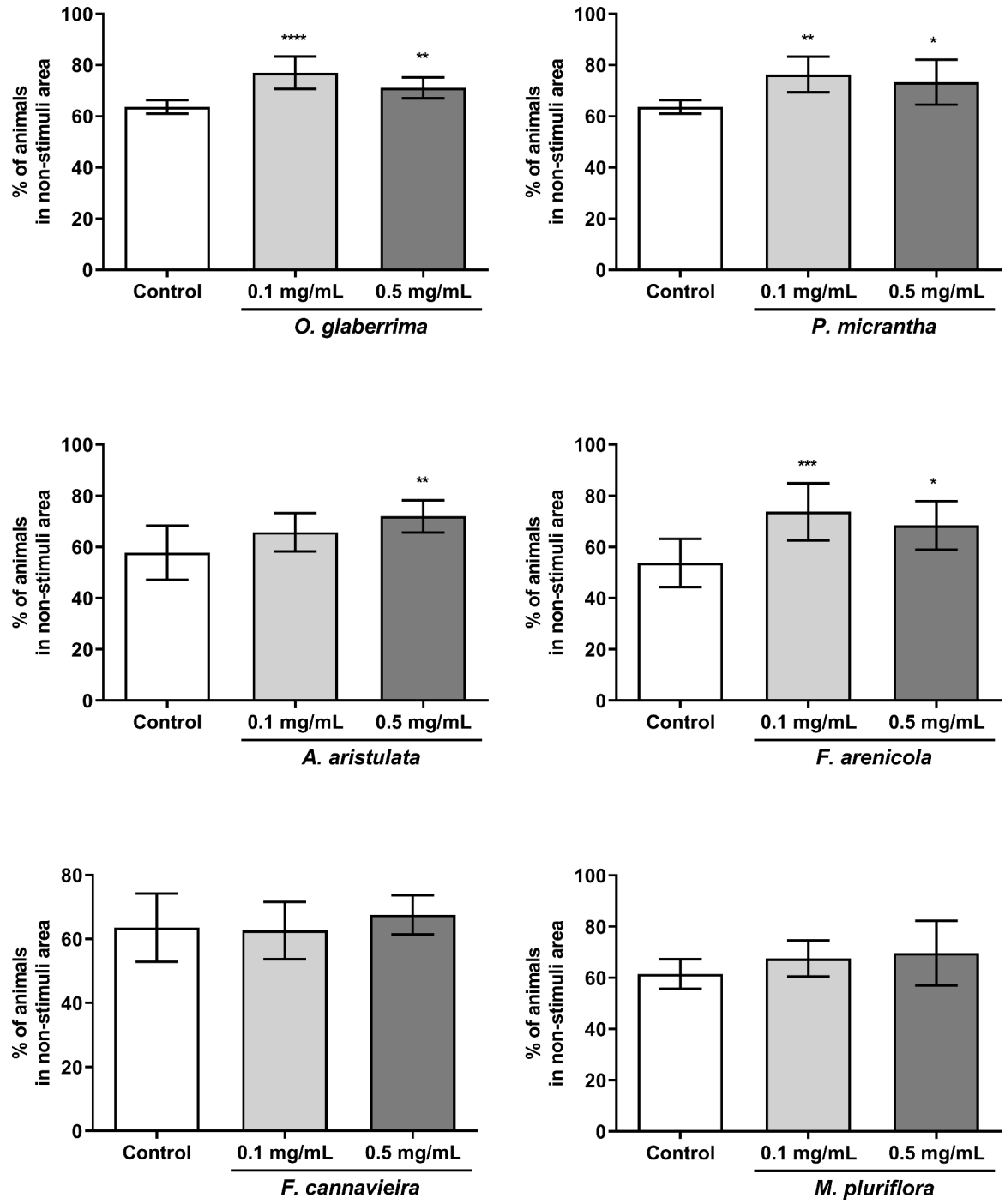

Fig. 6. . Percentage of the zebrafish larvae in non-stimuli area during avoidance behavior analysis after exposure to different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, PM, AA, FA, FC, MP (0.1 and 0.5 mg/ml): $n = 18$.

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) and pesticides (Altenhofen et al., 2019) on the exploratory behavior of zebrafish larvae exposed to such agents, and all of them observed a decrease in exploratory behavior caused by these compounds.

In the present study, it was also observed a decrease in the exploratory behavior of zebrafish larvae exposed to 0.5 mg/ml of bamboo extracts. Therefore, it was possible to infer that the bamboo extracts at 0.5 mg/ml caused a neurotoxic effect in the zebrafish larvae.

It is known that zebrafish larvae have an accurate vision, so studies have used projections on a 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 noticeably short latencies, but have surprising layers of complexity.

After exploratory analyses, larvae were submitted to avoidance-escape behavior from a visual stimulus (Table C.2). Treatments using both 0.1 mg/ml and 0.5 mg/ml concentrations of the OG ($p < 0.0001$; p

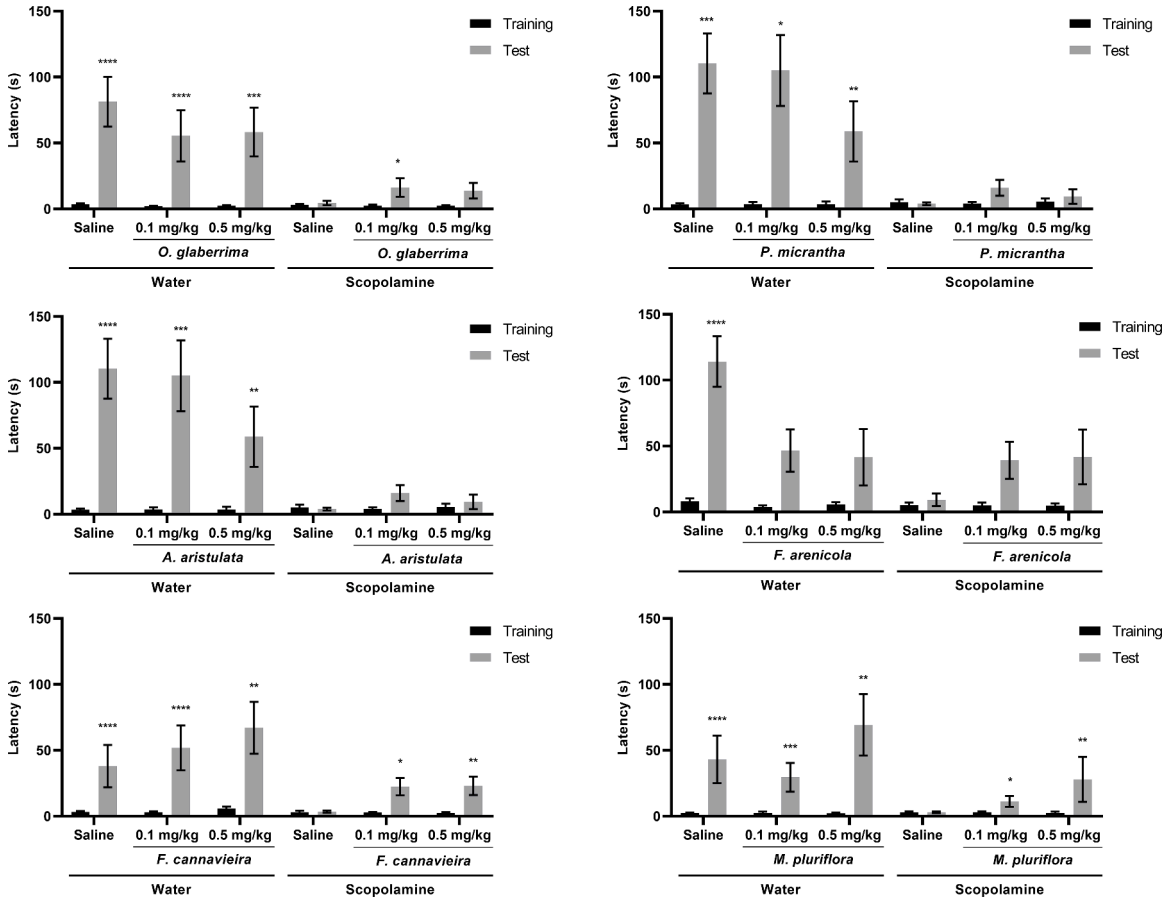

Fig. 7. . Latency (s) between training and test session of the animals on inhibitory avoidance analysis after exposure to different concentrations of Brazilian bamboo aqueous extracts. **** $p \leq 0.0001$; ** $p \leq 0.005$; * $p \leq 0.05$. OG, PM, AA, FA, FC, MP (0.1 and 0.5 mg/Kg): $n = 10$.

= 0.0063), PM ($p = 0.0014$; $p = 0.0141$), and FA ($p = 0.0008$; $p = 0.0131$) 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 AA extract ($p = 0.0038$) showed significant differences (Fig. 6).

Animals with their cognitive ability affected showed a decrease in the percentage of escaping responses from an aversive stimulus (Altenhofen et al., 2019). However, in the present study, an increase in escaping responses was observed for OG, PM, FA, and AA extracts, indicating an improvement of the cognitive capacity of the zebrafish larvae after the extract's administration.

The next step was to evaluate the performance of adult zebrafish in the inhibitory avoidance task (Table C.3). 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 (Fig. 7). 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 can reduce the memory deficits caused by scopolamine (Fig. 7).

The zebrafish inhibitory avoidance protocol proved to be efficient for studies of memory. 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).

Quercetin and rutin were tested using the zebrafish inhibitory avoidance protocol (Richetti et al., 2011). It was 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.

Conclusions

Brazilian bamboo species showed a great diversity of constituents, and many of them have never been described for bamboos. Although there are several studies on chemical characterization for Asian bamboo, many of them have focused on phenolic compounds, neglecting other classes of secondary metabolites. Furthermore, in this study, some of the detected compounds have no previous reports regarding their biological activity.

Aqueous extracts of the six Brazilian bamboo species showed high *in vitro* antioxidant potential, especially PM and FA. The extracts from OG, PM, AA, and FA increased the cognitive capacity of zebrafish larvae in the face of an aversive stimulus. Furthermore, OG, FC, and MP can reduce the memory loss induced by scopolamine in zebrafish adults. However, even though they are promising species, studies on the cultivation and reproduction of Brazilian bamboo species are required to further explore their potential in a sustainable process.

Author's contribution

JG, CMF, and FAM collected the plant material; JG prepared the extracts and conducted the *in vitro* experiments; JG and CMF designed the study; SA, DDN, and CDB designed the experiments with zebrafish; SA, DG, and JMKS conducted the experiments with zebrafish; LFY and MJK conducted the HPLC-ESI-MS/MS analysis; JG wrote the manuscript; CMF, FAM, CDB, SA, LFY, and MJK revised the manuscript. All authors approved the final version of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2021.100183.

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