Bioorganic Chemistry 91 (2019) 103122

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Phytoscreening of Vochysiaceae species: Molecular identification by HPLC-ESI-MS/MS and evaluating of their antioxidant activity and inhibitory potential against human α-amylase and protein glycation

R.R. Franco[a], A.B. Justino[a], M.M. Martins[a], C.G. Silva[b], P.R.V. Campana[b], J.C.D. Lopes[c], V.L. De Almeida[b], F.S. Espindola[a,*]

[a] Instituto de Biotecnologia (IBTEC), Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil
[b] Serviço de Fitoquímica e Prospecção Farmacêutica, Fundação Ezequiel Dias, Belo Horizonte, 30510-010, MG, Brazil
[c] Departamento de Química, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil

ARTICLE INFO

Keywords:
Postprandial hyperglycemia
Plant antioxidants
Advanced glycation end products
Polyphenols

ABSTRACT

Scientific research based on medicinal plants has been highlighted as a complementary treatment to T2DM, stand out the Vochysiaceae family, which have been widely used in folk medicine by traditional South American communities to treat some diseases. Our study aimed to investigate the antioxidant and antiglycation activities of ethanol extracts of leaves (LF) and stem barks (SB) of Vochysiaceae species, evaluated their capacities to inhibit glycoside and lipid hydrolases related to T2DM and molecular identification by HPLC-ESI-MS/MS. Our main findings indicate that the ethanolic extract of four of eight analyzed plants such as LF and SB of *Q. grandiflora*, *Q. parviflora*, *V. elliptica* and *Calisthene major* exhibited, respectively, potential of α-amylase inhibition (IC_{50} of LF: 5.7 ± 0.6, 4.1 ± 0.5, 5.8 ± 0.5, 3.2 ± 0.6 and IC_{50} of SB: 3.3 ± 0.7, 6.2 ± 2.0, 121.0 ± 8.6 and 11.2 ± 2.8 μg/mL), capacities of antioxidant (ORAC of LF: 516.2 ± 0.1, 547.6 ± 4.9, 544.3 ± 6.1, 442.6 ± 2.4 and ORAC of SB: 593.6 ± 22.3, 497.7 ± 0.8, 578 ± 12.3, 593.6 ± 19.5 μmol trolox eq/g; FRAP of LF: 796.1 ± 0.9, 427.7 ± 22.0, 81.0 ± 1.9, 685 ± 37.9 and FRAP of SB: 947.4 ± 24.9, 738.6 ± 24.3, 98.8 ± 7.9, 970.8 ± 13.9 μmol trolox eq/g; DPPH IC_{50} of LF: 14.2 ± 1.8, 36.3 ± 6.9, 11.8 ± 1.9, 13.3 ± 1.2 and DPPH IC_{50} of SB: 16.0 ± 3.0, 15.5 ± 1.9, 126.1 ± 23. 6, 5.3 ± 0.3 μg/mL, respectively) and antiglycation (BSA/Frutose IC_{50} of LF: 43.1 ± 3.4, 52.1 ± 6.0, 175.5 ± 32, 8, 111.8 ± 14.7 and BSA/Frutose IC_{50} of SB:, 40.1 ± 11.9, 51.2 ± 16. 7, 46.6 ± 5.7, 53.5 ± 13.6 μg/mL) and presence of polyphenols, such as flavonoids and condensed tannins. The extracts presented low ability to inhibit α-glycosidase and lipase enzymes in the initial assays, with values below 40% of inhibition. In BSA/methylglyoxal, only *Q. grandiflora* SB, *V. eliptica* LF and *V. tucanorum* LF showed activity (IC_{50}: 655.5 ± 208.5, 401.9 ± 135.2 and 617.1 ± 80.6 μg/mL, respectively) and only *C. major* LF and SB, in Arg/methylglyoxal (IC_{50}: 485.1 ± 130.8 and 468.0 ± 150.5 μg/ml, respectively). This study presented new findings about the biological and pharmacological potential of some species of Vochysiaceae family, contributing to the understanding of the action and efficacy in use of these plants, in their management of postprandial hyperglycemia and in glycation and oxidative processes that contribute to managing diabetes *mellitus*.

1. Introduction

The World Health Organization stipulates that the pathology of diabetes mellitus affects about 422 million people worldwide [1]. The type 2 of diabetes mellitus (T2DM) is an endocrine disorder characterized by muscle resistance to the action of insulin, which leads to metabolic dysfunctions, such as hyperglycemia and hyperlipidemia in fasting and postprandial periods [2]. The high concentration of sugars and lipids in human serum stimulates the excessive formation of reactive oxygen species (ROs), protein glycation and inflammation [3].

The search of new pharmacological alternatives in the treatment of T2DM stimulates the study with natural products, such as medicinal plants [4]. Research with medicinal plants has demonstrated the increasing antidiabetic potential of flavonoids, condensed tannins and other polyphenols, which are widely distributed in plants [5]. Among the medicinal plants, we can mention the species of the family

* Corresponding author at: Universidade Federal de Uberlândia, Instituto de Biotecnologia, Av. Pará, 1720, CEP 38400-902, Uberlândia, MG, Brazil.
E-mail address: foued@ufu.br (F.S. Espindola).

https://doi.org/10.1016/j.bioorg.2019.103122
Received 21 December 2018; Received in revised form 29 June 2019; Accepted 11 July 2019
Available online 12 July 2019
0045-2068/

Vochysiaceae which have been widely used in folk medicine by traditional South American communities to treat some diseases [6–8]. According to Neto et al. (2011), several studies have determined the phytochemical constitution and the medicinal potential of Vochysiaceae species, in special *Qualea* spp., *Vochysia* spp., *Callisthene* spp. and *Salvertia* spp. genera.

According to the literature, infusions and decoctions prepared with leaves of *Qualea grandiflora* Mart. are indicated to treat bloody diarrhea, intestinal colic [7] while their leaves and stem bark are indicated for treating ulcers [9], wounds and inflammatory diseases [10], and as an astringent [11]. According to Mazzolin et al. [12], the methanolic extract of the leaves of *Qualea parviflora* Mart. showed antihemorrhagic activity and low mutagenic effect. In addition, the methanolic extract from its barks had the ability to inhibit α-amylase [13].

According to Gomes et al. (2009), the methanolic extract of the leaves of *Vochysia tucanorum* Mart., as well as its *n*-butanolic fraction, presented excellent gastroprotection activity, antioxidant capacity and NO synthesis and showed no signs of cytotoxicity. In previous studies, our group indicated that the aqueous extract of stem bark of *Vochysia rufa* Mart. is able to reduce oxidative stress in liver [15] and pancreatic cells [16], contributing to hyperglycemia reduction and normalized the biochemical alterations found in diabetic rats [17]. In the study of Lopes et al. [18], two flavonoids were described in leaves of *Vochysia cinnamomea* Pohl., the 7,3′,4′-trihydroxyflavone and 3′-methoxy-7,4′-dihydroxyflavone, the latter compound being also found in *Salvertia convallariodora* St. Hil in the same work.

Vochysia elliptica is still little explored, but according to Cruz [19], the ethanolic extract of this plant showed low cytotoxicity and modest antioxidant activity. In the study of Castoldi et al. [20], the genus *Callisthene* spp. had no cytotoxic activity. In the bark of *Callisthene major* Mart. & Zucc, the ellagic acid and friedelin have been determinated by Corrêa et al. [21]. Although these plants have several therapeutic uses and part of their bioactive molecules has been evaluated, the antidiabetic effects are not fully described. The aim of this study was to investigate some ethanolic extracts of species from the Vochysiaceae family, to identify their main phytochemicals constituents and to evaluate their antidiabetic effects in antioxidant capacity assays and inhibitory activities against α-amylase, α-glycosidase, pancreatic lipase and non-enzymatic glycation.

2. Material and methods

2.1. Standards chemicals

Solvents, reagents and standards were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Plant material

The plants were selected based on their general pharmacological and ethnobotanical studies on the family Vochysiaceae (Table 1). The species *Q. grandiflora*, *V. elliptica*, *Q. parviflora*, *S. convalliodora*, *C. major*, *V. tucanorum* and *V. cinnamomea* were obtained through a partnership with the Phytochemical laboratory of Ezequiel Dias Foundation (Belo Horizonte, Minas Gerais, Brazil) and were collected in the *Cerrado* area of Minas Gerais, and our study group, situated in Federal University of Uberlandia, collected the *V. rufa* in the same biome. All plant samples were deposited in the herbaria of each institution. Voucher specimens of material collected by the group from the Phytochemical laboratory were deposited in the Herbarium PAMG of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). This study received authorization for access and remittance of genetic material for scientific research by SISGEN (n° A933BED).

Table 1
Botanical identification of Vochysiaceae species used in this study.

Scientific name	Parts of plants*	Voucher from herbaria	Refs.
Q. grandiflora	Stem bark and Leaves	PAMG 52,469	[22]
V. elliptica	Stem bark and Leaves	PAMG 52,492	[19]
Q. parviflora	Stem bark and Leaves	PAMG 52,496	[11]
S. convalliodora	Leaves	PAMG 52,497	[23]
C. major	Stem bark and Leaves	PAMG 53,154	[24]
V. tucanorum	Stem bark and Leaves	PAMG 56,277	[14]
V. cinnamomea	Leaves	PAMG 57,015	[25]
V. rufa	Stem bark	HUFU 58,888	[15]

Note: * parts of plants selected for this study.

2.3. Preparation of extracts

The stem bark of *V. rufa* was crushed and subjected to static maceration for seven days, using 500 g of plant material for each 2.5 L of 98% ethanol (1:5 m/v). After extraction, the solutions were filtered and the solvent completely removed by rotary evaporator under reduced pressure at 40 °C. The maceration process was repeated three times to warranty extraction exhaustion, and extracts were frozen and lyophilized to remove remaining water. The other species were separated manually and dried in an oven with air circulation at 40 °C and powdered. The extracts were prepared with 10 g of each plant material for every 300 mL of 98% ethanol (1:30 m/v). The solutions were transferred for an ultrasonic bath at 40 KHz for 10 min; filtered and concentrated using a rotary evaporator. The procedure was repeated three times, and the combined extracts were dried and stored in a freezer at −20 °C.

2.4. Phytochemical prospection assays

The phytochemical prospecting methods were based on Justino et al. [26]. For determination of total content of condensed tannins, the extracts were diluted in methanol at a concentration of 100 μg/mL and at 500 μg/mL for determination of phenolic and flavonoid contents.

2.4.1. Determination of total phenolic content

The extracts were mixed with Folin-Ciocalteu solution 10% (v/v) and Na_2CO_3 solution 7.5% (m/v). The mixture was incubated at 50 °C for 5 min and the absorbance was read at 760 nm (Thermo Scientific, Genesys 10S UV–Vis, USA). The total phenolic content was determined using an analytical curve, constructed with gallic acid as standard (mg GAE g^{-1}).

2.4.2. Determination of total condensed tannins content

The extracts were mixed with vanillin 0.5% (m/v) in H_2SO_4 solution 70% (m/v). The mixture was incubated at 50 °C for 15 min and the absorbance was read at 500 nm (Thermo Scientific, Genesys 10S UV–Vis, USA). The condensed tannins content was determined using an analytical curve, constructed with catechin as standard (mg CE/g).

2.4.3. Determination of total flavonoid content

The extracts were mixed with methanol solution of $AlCl_3$ 5% (m v^{-1}). The mixture was incubated at room temperature for 30 min and the absorbance was measured at 425 nm (Thermo Scientific, Genesys 10S UV–Vis, USA). The flavonoid content was obtained using an analytical curve, constructed with quercetin as standard (mg QE g^{-1}).

2.5. Enzymatic assays

The enzymatic assays were based on Franco et al. (2018). The extracts, quercetin (pure compound identified) and positive controls (acarbose, for α-amylase/α-glucosidase and Orlistat for lipase) were diluted in dimethyl sulfoxide (DMSO) and used at a concentration of 10 mg/mL for initial assays. The samples have been diluted serially

from a concentration of 30 mg/mL for IC_{50} determination. All analyzes were performed in triplicate and results are presented as percentage (%) of inhibition, calculated using the following equation:

$$I(\%) = [(A\ \ control - A\ \ sample)/(A\ \ control)] \times 100$$

where *A control* is the absorbance value of the negative control and *A sample* is the absorbance value of each extract/positive control.

2.5.1. α-amylase inhibition

The samples were analyzed for inhibition of α-amylase activity using 2-chloro-4-nitrophenyl-4-β-D-galactopyranosylmaltoside (GalG2CNP) as substrate and a saliva fraction enriched with α-amylase (HSA-f). The HAS-f was diluted in 50 mM of 2-(Nmorpholino)-ethanesulfonic acid (MES) buffer containing 5 mM of calcium chloride, 140 mM of potassium thiocyanate and 300 mM of sodium chloride (pH 6.0). Each extract was incubated with HSA-f (1:10 ratio) for 30 min at 37 °C. Subsequently, 12 mM of GALG2CNP was added to initiate the reaction. Increases in absorbance (i.e., CNP release) were measured at 37 °C for 3 min and at 405 nm (Molecular Devices, Menlo Park, CA, USA).

2.5.2. α-glucosidase inhibition

The samples were analyzed for their ability to inhibit α-glucosidase activity using a method with 4-nitrophenyl α-D-glucopyranoside (p-NPG) as substrate and α-glucosidase-enriched fraction from rat's intestinal acetone powder (AG-f). Each extract was incubated with AG-f and 1.5 mM reduced glutathione (diluted in 50 mM phosphate buffer pH 6.8) for 20 min at 37 °C. The reaction started by adding 4 mM p-NPG and the absorbance values were measured at 405 nm (Molecular Devices, Menlo Park, CA, USA) for 30 min. Phosphate buffer was used as negative control.

2.5.3. Lipase inhibition

The samples were analyzed for their ability to inhibit lipase activity using a modified method with p-nitrophenyl palmitate (p-NPP) as substrate and porcine pancreatic lipase. Each extract was incubated with 10 g/L PL (diluted in 50 mM Tris-HCl buffer pH 8.0, containing 10 mM $CaCl_2$ and 25 mM NaCl) for 20 min at 37 °C. The reaction started by adding 0.8 mM *p*-NPP substrate (diluted in 10% isopropanol and 50 mM Tris-HCl buffer, containing 10 mM $CaCl_2$, 25 mM NaCl, 0.5% triton X-100 and 0.1% gum arabic). The absorbance values were measured at 410 nm (Molecular Devices, Menlo Park, CA, USA) for 30 min. Tris-HCl buffer was used as negative control.

2.6. Antioxidant assays

The antioxidant assays were based on Justino et al. [27]. The extracts/ascorbic acid (positive control) were solubilized in ethanol at a concentration of 10 mg/mL for ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH) methods and at 100 μg/mL for the absorption capacity of oxygen radicals (ORAC) assay. The samples were serially diluted from a concentration of 30 mg/mL for DPPH IC_{50} determination. All analyzes were performed in triplicate and results were expressed as percentage (%) of antioxidant capacity for DPPH assay, and trolox equivalents (μmol TE/g) for FRAP and ORAC assays.

2.6.1. Sequestration of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The extracts/ascorbic acid were incubated with a methanol solution of 60 mM DPPH at 30 °C for 20 min, in absence of light. The reduction in absorbance of the mixture was measured at 517 nm. Scavenging of DPPH radicals was calculated using the following equation $DPPH(\%) = [(A\ \ control - A\ \ sample)/(A\ \ control)] \times 100$, where *A control* is the absorbance value of the DPPH radical and *A sample* is the absorbance value of each extract/positive control. Methanol was used as negative control.

2.6.2. Oxygen radical absorbance capacity (ORAC)

The extracts/ascorbic acid were incubated with 0.085 nM fluorescein at room temperature for 15 min. After incubation, 153 mM 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) was added and the fluorescence (485 nm_{ex}/528 nm_{em}) was measured at 37 °C for 90 min. The loss of fluorescence was measured by calculating the area under the curve. All reagents were prepared in 75 mM phosphate buffer (pH 7.4). The antioxidant capacity was determined using an analytical curve, constructed with trolox as standard. Phosphate buffer was used as negative control.

2.6.3. Iron reduction capacity (FRAP)

The extracts/ascorbic acid were incubated with 10 volumes of 300 mM sodium acetate buffer (pH 3.6), 1 vol of 10 mM 2,4,6-tri (2pyridyl)-striazine (TPTZ) and 1 vol of 20 mM ferric chloride at 37 °C for 6 min. Absorbance values were measured at 593 nm and the antioxidant capacity was determined by an analytical curve constructed with trolox. Sodium acetate buffer was used as negative control.

2.7. Inhibition test of advanced glycation end products (AGEs) formations

The Inhibition test of AGEs formations were based on Wang et al. [28]. The extracts/quercetin (positive control) were diluted in DMSO and used at a concentration of 10 mg/mL and serially diluted from a concentration of 30 mg/mL for IC_{50} determination in these methods. All analyzes were performed in triplicate and results are presented as percentage (%) of inhibition, calculated using the following equation: $GI(\%) = [(F\ \ control - F\ \ sample)/(F\ \ control)] \times 100$, where *F control* is the fluorescence value of the negative control and *F sample* is the fluorescence value of each extract/quercetin. Phosphate buffer was used as negative control.

2.7.1. Bovine serum albumin and fructose method (BSA/FRU)

The extracts and control were incubated with 50 mg/mL BSA and 1.25 M fructose (diluted in 200 mM phosphate buffer, pH 7.4 containing 0.02% sodium azide) at 37 °C for 72 h in the dark. After incubation, 20% trichloroacetic acid (m/v) was added and the mixture was centrifuged at 10,000 for 10 min. The Pellet was resuspended in phosphate buffer and fluorescence intensity of glycated albumin was measured (350 nm_{ex}/420 nm_{em}).

2.7.2. Bovine serum albumin and methylglyoxal method (BSA/MGO)

The extracts and controls were incubated in the dark with 50 mg mL^{-1} BSA (diluted in 200 mmol L^{-1} phosphate buffer, pH 7.4, with 0.02% sodium azide) and 53.3 mmo L^{-1} methylglyoxal (diluted in deionized water) at 37 °C for 72 h. Then, 20% trichloroacetic acid (m v^{-1}) was added and the mixture was centrifuged at 10,000 xg for 10 min. The pellet was resuspended in phosphate buffer and the fluorescence intensity (340 nm_{ex}/380 nm_{em}) was measured. Quercetin was used as a positive control and 5% DMSO as a negative control. The blank was carried substituting methylglyoxal by phosphate buffer.

2.7.3. Arginine and methylglyoxal method (Arg/MGO)

The extracts were incubated in the dark with 106.6 mmol L^{-1} arginine (diluted in 200 mmol/L phosphate buffer, pH 7.4, with 0.02% sodium azide) and 53.3 mmol/L methylglyoxal (diluted in deionized water) at 37 °C for 72 h. Then, the fluorescence intensity (340 nm_{ex}/380 nm_{em}) was measured. Quercetin was used as a positive control and 5% DMSO as a negative control. The blank was carried substituting methylglyoxal by phosphate buffer.

2.8. Liquid chromatography-mass spectrometry analysis

The compounds in the fractions which showed better biological activities were identified by high-performance liquid chromatography coupled to a mass spectrometer with electrospray ionization (HPLC-ESI-

MS/MS, Agilent Q-TOF, model 6520). MeOH–H_2O (4:1) was used as solvent system and the samples were infused into the ESI source at a flow rate of 200 µL/h. Nitrogen gas was used as drying gas at a flow rate of 8 L/min and as nebulizer gas at 58 psi. The temperature of nebulizer was set at 200 °C and a potential of 4.5 kV was applied in the capillary. The electron impact energy was set at 10–30 eV. The HPLC parameters were: Agilent Zorbax model 50 × 2.1 mm column, particles of 1.8 µm and pore diameter of 110 Å, mobile phase: water acidified with formic acid (0.1% v/v) (A) and methanol (B). The gradient solvent system (B) was: 2% (0 min); 98% (0–15 min); 100% (15–17 min); 2% (17–18 min); 2% (18–22 min), with a flow of 0.35 mL/min and detection at UV of 280 and 360 nm. The data were acquired in negative and positive modes, with adjustment for a range of 20–1000 m/z. The mass spectrometry data were evaluated using the Agilent Mass Hunter B.07.00 software and the molecular ions and their fragments were compared with results from other studies in literature. Based on the error calculated from the theoretical and observed m/z values (in this case we used error ≤ 10), we suggest the compounds presents in Vochysiaceae species.

2.9. Statistical analysis

The statistical analyzes and graphics were done using GraphPad Prism 6.0 software. The data was expressed as mean ± standard error of the mean (SEM) and the significance of difference has been calculated using one-way ANOVA and Tukey post-test of multiple comparisons. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Phytochemical prospection

The results of the phytochemicals prospecting trials with leaf (LF) extracts and stem bark (SB) of the Vochysiaceae species are shown in Table 2. In the quantification of phenolic content, extracts of leaves and stem bark of *Q. grandiflora*, *Q. parviflora* and *C. major* showed values higher than 210 mg GAE/g, while the other extracts presented values close to or less than 110 mg GAE/g. With regard to the quantification of the flavonoids content, extracts of LF of *Q. grandiflora*, *C. major* and *V. tucanorum* showed values higher than 41 mg QE/g, practically double the other extracts, which presented equal or lower values to 21 mg QE/g. The content of condensed tannins was low in most of the extracts tested, with values close to 20 mg CE/g, however the extract of *C. major* SB presented a value greater than 60 mg CE/g.

Table 2
Evaluation of total polyphenols, flavonoids and condensed tannins content in ethanolic extracts of Vochysiaceae species.

Extracts	Phenolic (mg GAE/g)	Flavonoids(mg QE/g)	Condensed tannins (mg CE/g)
Q. grandiflora LF	254.4 ± 2.7^{a}	65.5 ± 0.3^{e}	12.6 ± 0.8^{a}
Q. grandiflora SB	316.9 ± 9.3^{e}	11.1 ± 0.2^{e}	19.4 ± 1.5^{b}
V. elliptica LF	84.2 ± 0.4^{b}	8.2 ± 0.7^{a}	14.6 ± 0.1^{a}
V. elliptica SB	78.0 ± 0.3^{b}	3.0 ± 0.1^{b}	7.7 ± 2.5^{c}
Q. parviflora LF	212.8 ± 3.7^{e}	21.0 ± 0.3^{e}	5.6 ± 1.0^{cd}
Q. parviflora SB	275.7 ± 0.3^{e}	7.8 ± 0.1^{a}	13.1 ± 0.1^{a}
S. convalliodora LF	136.9 ± 1.2^{e}	17.7 ± 1.2^{e}	7.0 ± 0.4^{ce}
C. major LF	250.7 ± 4.5^{a}	41.7 ± 0.5^{e}	9.6 ± 0.7^{ac}
C. major SB	352.6 ± 2.3^{e}	8.7 ± 0.2^{a}	64.9 ± 2.4^{g}
V. tucanorum LF	72.1 ± 0.7^{bc}	50.3 ± 0.6^{e}	12.1 ± 1.1^{a}
V. tucanorum SB	43.5 ± 0.5^{d}	1.6 ± 0.3^{b}	7.3 ± 0.7^{cf}
V. cinnamomea LF	108.9 ± 3.7^{e}	14.1 ± 0.2^{e}	20.2 ± 0.4^{b}
V. rufa SB	57.1 ± 0.4^{cd}	1.8 ± 0.1^{b}	3.8 ± 1.5^{def}

Note: Ethanolic extracts of Leaves (LF) and Stem bark (SB) of Vochysiaceae species. All extracts were diluted in methanol, at a concentration of 500 µg/mL, for the determination of phenolic and flavonoids content, and 100 µg/mL, for the determination of proanthocyanidins content. Values expressed as mean ± standard error. Gallic acid (GAE), quercetin (EQ) and catechin (CE) were used for make standards curves in total content of phenolic, flavonoids and proanthocyanidins, respectively. Different letters indicate significant difference ($p < 0.05$).

Table 3
Evaluation of the antioxidant capacity of ethanolic extracts of Vochysiaceae species using DPPH, ORAC and FRAP methods.

Samples	DPPH (%)	ORAC (µmol trolox eq/g)	FRAP (µmol trolox eq/g)
Q. grandiflora LF	90.5 ± 0.7^{a}	516.2 ± 0.1^{a}	796.1 ± 0.9^{d}
Q. grandiflora SB	97.4 ± 0.1^{b}	593.6 ± 22.3^{b}	947.4 ± 24.9^{a}
V. elliptica LF	96.0 ± 0.1^{b}	544.3 ± 6.1^{ac}	81.0 ± 1.9^{b}
V. elliptica SB	91.7 ± 0.6^{a}	578 ± 12.3^{bcd}	98.8 ± 7.9^{b}
Q. parviflora LF	93.2 ± 0.4^{c}	547.6 ± 4.9^{ad}	427.7 ± 22.0^{e}
Q. parviflora SB	99.6 ± 0.2^{d}	497.7 ± 0.8^{a}	738.6 ± 24.3^{f}
S. convalliodora LF	98.1 ± 0.1^{b}	682.9 ± 2.5^{e}	249.0 ± 1.3^{g}
C. major LF	94.1 ± 0.1^{c}	442.6 ± 2.4^{f}	685 ± 37.9^{h}
C. major SB	93.9 ± 0.2^{c}	593.6 ± 19.5^{b}	970.8 ± 13.9^{a}
V. tucanorum LF	80.6 ± 0.5^{e}	307.8 ± 3.3^{g}	23.2 ± 0.3^{c}
V. tucanorum SB	83.6 ± 1.3^{f}	566.1 ± 7.1^{bcd}	20.4 ± 1.6^{c}
V. cinnamomea LF	90.1 ± 0.1^{a}	913.7 ± 27.1^{h}	170.2 ± 11.9^{i}
V. rufa SB	89.7 ± 0.3^{a}	621.2 ± 14.7^{b}	57.5 ± 0.7^{bc}
Ascorbic acid	100 ± 0^{d}	2577 ± 0.3^{i}	1317 ± 6.5^{j}
Quercetin	100 ± 0^{d}	4047 ± 89.5^{i}	1342 ± 5.3^{j}

Note: Ethanolic extracts of Leaves (LF) and Stem bark (SB) of Vochysiaceae species. All extracts, quercetin and positive control were diluted in EtOH at a concentration of 10 mg/mL. Values (mean ± standard error) expressed as a percentage of antioxidant capacities (DPPH) and µmol trolox equivalents/g (ORAC and FRAP). Ascorbic acid was used as a control for these methods. Different letters indicate significant difference ($p < 0.05$).

3.2. Antioxidant assays

The results of the antioxidant assays with LF and SB extracts of the Vochysiaceae species are shown in Table 3. By the ORAC method, the extract *V. cinnamomea* LF (913.7 ± 27.1 µmol trolox eq/g) presented the closest result obtained by ascorbic acid (2577 ± 0.3 µmol trolox eq/g) and quercetin (4047 ± 89.5 µmol trolox eq/g), while the other extracts showed lower activities, from 300 µmol trolox eq/g. In the DPPH method, all the extracts presented high antioxidant capacity, presenting values above 80%. From the FRAP method, it was possible to observe that extracts of LF and SB of plants *Q. grandiflora*, *Q. parviflora* and *C. major* showed the highest values of antioxidant capacity, with values higher than 400 µmol trolox eq/g, whereas that the other extracts presented values below 250 µmol trolox eq/g. However, the pure compound quercetin presented high antioxidant capacity by the FRAP method (1342 ± 5.3 µmol trolox eq/g).

The results of the half maximal inhibitory concentration (IC_{50}) values of leaves and stem bark extracts of the Vochysiaceae species with more 50% of DPPH activity with are shown in Fig. 1. The extracts and quercetin (3.3 ± 0.4 µg/mL) showed no significant differences among them and with ascorbic acid (6.9 ± 0.8 µg/mL). Extracts from the LF of *Q. grandiflora*, *V. eliptica*, *Q. parviflora*, *C. major*, *V. tucanorum*, *S. convalliodora* and *V. cinnamomea* presented IC_{50} of 14.2 ± 1.8, 11.8 ± 1.9, 36.3 ± 6.9, 13.3 ± 1.2, 125.0 ± 77.6, 82.9 ± 13.2 and 25.1 ± 3.5 µg/mL, respectively. On the other hand, the SB of *Q. grandiflora*, *V. elliptica*, *Q. parviflora*, *C. major*, *V. tucanorum* and *V. rufa* showed IC $_{50}$ of: 16.0 ± 3.0, 126.1 ± 23. 6, 15.5 ± 1.9, 5.3 ± 0.3, 164.0 ± 19.6 and 6.6 ± 0.6 µg/mL, respectively.

3.3. Antiglycation assays

The results of the antiglycation assays with LF and SB extracts of the Vochysiaceae species are shown in Table 4. In all assays, quercetin showed 100% of antiglycation activity. By the BSA/FRU method, all the extracts presented antiglycation capacity, with average values above 94% of activity. In the other hand, few extracts presented activity in the

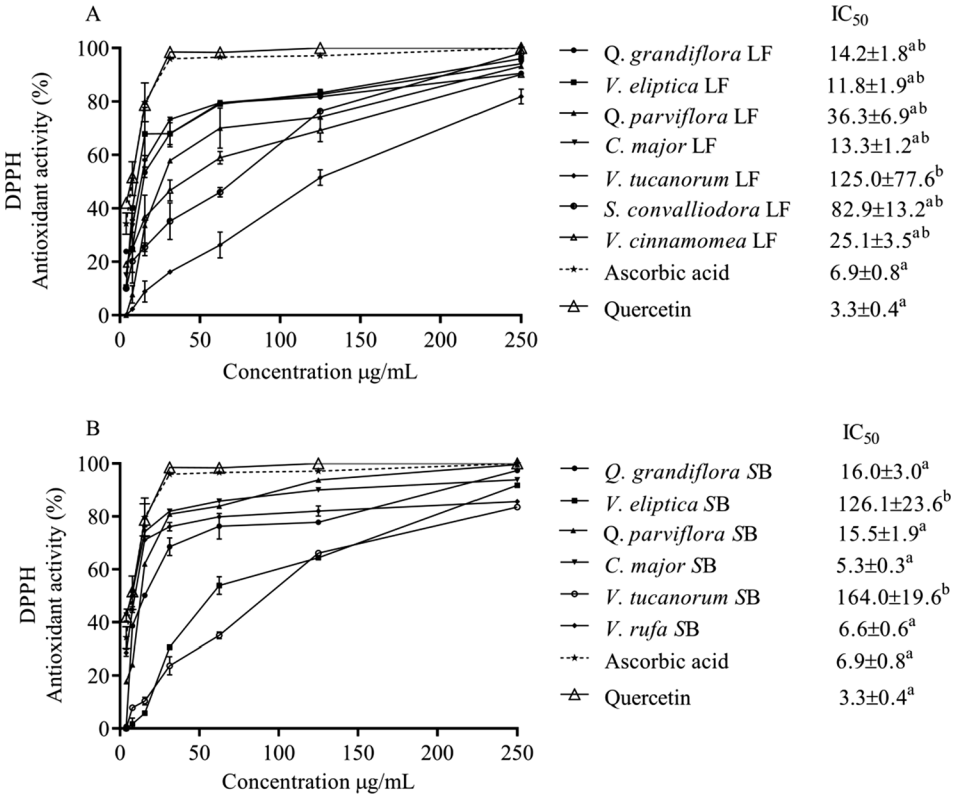


Fig. 1. Half maximal inhibitory concentration (IC_{50}) values of extracts with more 50% of DPPH activity. All extracts, quercetin and positive control were serially diluted in EtOH at a concentration of 30 mg/mL. Note: (A) Ethanolic extracts of Leaves (LF) and (B) Stem bark (SB) of Vochysiaceae species. Ascorbic acid was used as a control for DPPH method. Different letters indicate significant difference ($p < 0.05$).

Table 4
Evaluation of the antiglycation capacity of ethanolic extracts of Vochysiaceae species using BSA/Fructose, BSA/Methylglyoxal and Arginine/Methylglyoxal methods.

Samples	BSA/FRU (%)	BSA/MGO (%)	ARG/MGO (%)
Q. grandiflora LF	100 ± 0[a]	NA	43.9 ± 0.1[a]
Q. grandiflora SB	100 ± 0 [a]	57.8 ± 1.9[a]	48.4 ± 0.1[b]
V. elliptica LF	97.9 ± 0.1[b]	66.4 ± 0.1[c]	NA
V. elliptica SB	98.1 ± 0.3[b]	4.9 ± 0.1[d]	NA
Q. parviflora LF	100 ± 0 [a]	NA	NA
Q. parviflora SB	100 ± 0 [a]	14.3 ± 0.5[e]	45.2 ± 0.2[c]
S. convalliodora LF	98.4 ± 0.1[b]	40.7 ± 1.0[b]	NA
C. major LF	99.9 ± 0.1 [a]	NA	54.7 ± 0.2[d]
C. major SB	100 ± 0 [a]	42.1 ± 0.4[b]	57.3 ± 0.1[e]
V. tucanorum LF	98.3 ± 1.2[b]	59.7 ± 0.1 [a]	NA
V. tucanorum SB	91.9 ± 0.2[c]	25.5 ± 0.2[f]	NA
V. cinnamomea LF	100 ± 0 [a]	NA	NA
V. rufa SB	94.5 ± 0.1[d]	34.6 ± 0 [g]	19.4 ± 0.03[f]
Quercetin	100 ± 0 [a]	100 ± 0 [h]	100 ± 0 [g]

Note: Ethanolic extracts of Leaves (LF) and Stem bark (SB) of Vochysiaceae species. All extracts and quercetin were serially diluted in DMSO at a concentration of 10 mg/mL. Values (mean ± standard error) expressed as a percentage of antiglycation in the Bovine serum albumin/Fructose (BSA/FRU), BSA/Methylglyoxal (BSA/MGO) and Arginina/ Methylglyoxal (Arg/MGO). Quercetin was used as a control for these methods. Different letters indicate significant difference ($p < 0.05$).

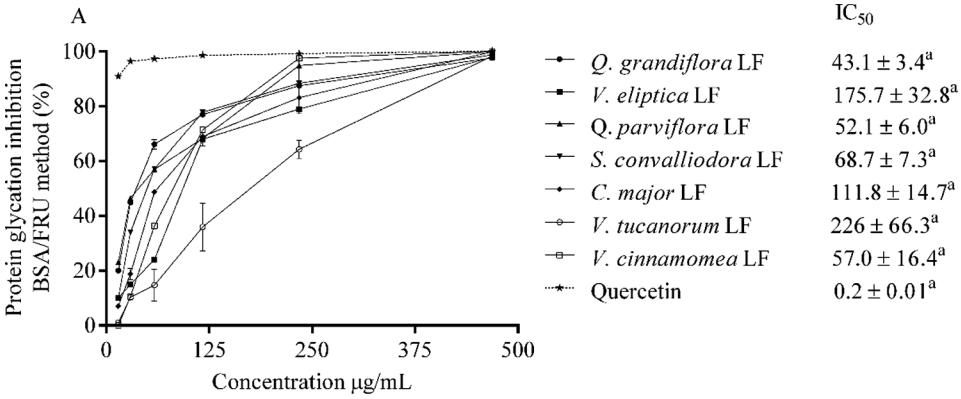
BSA/MGO method, and the extracts *Q. grandiflora* SB, *V. elliptica* LF and *V. tucanorum* LF were the only ones that presented more than 50% of activity, presenting the following values: 57.8 ± 1.9, 66.4 ± 0.1 and 59.7 ± 0.1 respectively. Only LF and SB of *C. major* presented more than 50% activity in the ARG/MGO method, with respective values of 54.7 ± 0.1 and 57.3 ± 0.1.

The Fig. 2 shows the results of IC_{50} values from antiglycation capacity in the BSA/FRU assay using the ethanolic extracts of the Vochysiaceae species, which presented more than 50% of activity. The extracts presented values without significant difference among themselves and with quercetin (0.2 ± 0.01 µg/mL). The IC_{50} obtained for the leaves of *Q. grandiflora*, *V. elliptica*, *Q. parviflora*, *S. convalliodora*, *C. major*, *V. tucanorum* and *V. cinnamomea* were 43.1 ± 3.4, 175.5 ± 32.8, 52.1 ± 6.0, 68.7 ± 7.3, 111.8 ± 14.7, 226 ± 66.3 and 57.0 ± 16.4 µg/mL, respectively. In the other hand, the IC_{50} obtained for the SB of *Q. parviflora*, *V. elliptica*, *Q. grandiflora*, *C. major*, *V. tucanorum* and *V. rufa* were 46.6 ± 5.7, 51.2 ± 16.7, 40.1 ± 11.9, 53.5 ± 13.6, 154 ± 33.6 and 87.5 ± 33.5 µg/ml.

The Fig. 3 shows the results of IC_{50} values from antiglycation capacity in the BSA/MGO and Arg/MGO assays using the ethanolic extracts of Vochysiaceae species and quercetin, which presented more than 50% of activities. In BSA/MGO IC_{50}, the results obtained for quercetin was 48.5 ± 3.9 µg/mL and for *Q. grandiflora* SB, *V. eliptica* LF and *V. tucanorum* LF were 655.5 ± 208.5, 401.9 ± 135.2 and 617.1 ± 80.6 µg/mL, respectively. On the other hand, the IC_{50} obtained for quercetin was 190 ± 29.4 µg/mL and for *C. major* LF and SB were 485.1 ± 130.8 and 468.0 ± 150.5 µg/ml.

3.4. Enzyme assays

The results of the enzymes inhibitions, using LF and SB extracts of Vochysiaceae species, are shown in Table 5 and presented as percent enzymatic inhibition. The acarbose was able to inhibit respectively 100 ± 0% and 71.4 ± 1.8% of the α-amylase and α-glucosidase enzymes respectively. In addition, orlistat inhibited the lipase enzyme by 100 ± 0%. In the α-amylase inhibition assay, extracts showed high inhibitory activity of this enzyme, especially *Q. grandiflora* LF (92.0 ± 3.5), *Q. grandiflora* SB (95.9 ± 1.0), *V. elliptica* LF (59.4 ± 9.1), *S. elliptica* SB (97.2 ± 2.8), *Q. parviflora* LF (84.8 ± 4.6), *Q. parviflora* SB (88.5 ± 4.3), *S. convalliodora* LF), *C. major* LF), *C major* SB (93.1 ± 3.6), *V. tucanorum* LF (44.6 ± 0.3), *V. tucanorum* SB (33.6 ± 0.3), *V. cinnamomea* LF (60.1 ± 7.8) and *V. rufa* SB (70.9 ± 8.2). On the other hand, the extracts tested showed results lower than 40% in the α-glycosidase inhibition assay, especially *Q. parviflora* SB (36.8 ± 3.3). In relation to the lipase enzyme inhibition



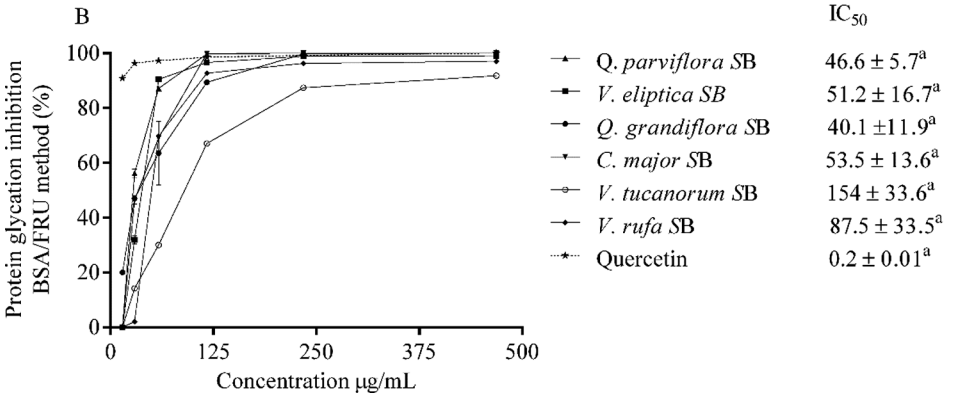
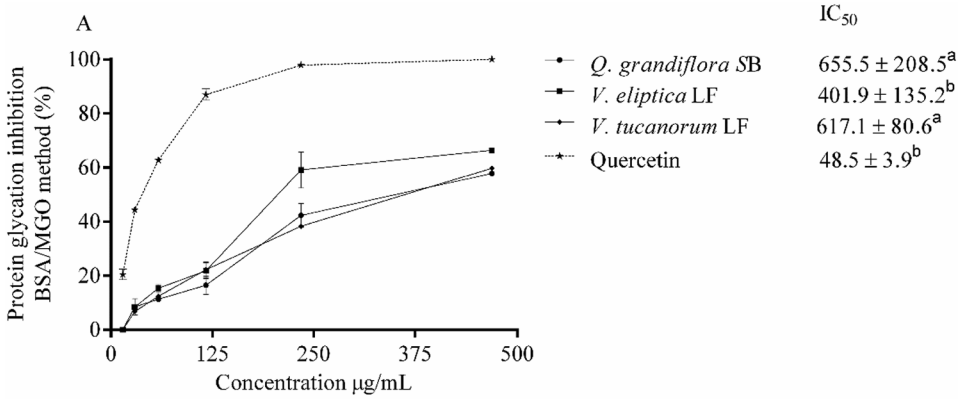


Fig. 2. Half maximal inhibitory concentration (IC_{50}) values of extracts with more 50% of BSA/Fructose activity. All extracts and quercetin were serially diluted in DMSO at a concentration of 30 mg/mL. Note: (A) Ethanolic extracts of Leaves (LF) and (B) Stem bark (SB) of Vochysiaceae species. Quercetin was used as a control. Different letters indicate significant difference ($p < 0.05$).



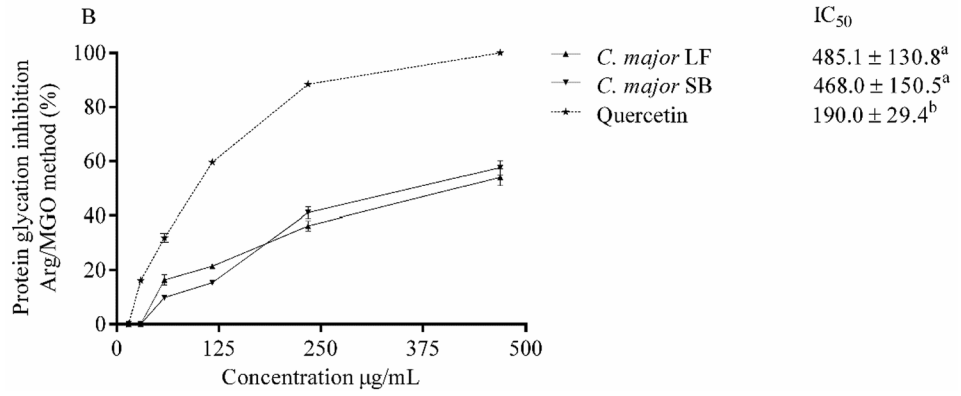


Fig. 3. Half maximal inhibitory concentration (IC_{50}) values of extracts with more 50% of (A) BSA/MGO and (B) Arg/MGO activities. All extracts and quercetin were serially diluted in DMSO at a concentration of 30 mg/mL. Note: (A) BSA/MGO method and (B) Arg/MGO method. Quercetin was used as a control. Different letters indicate significant difference ($p < 0.05$).

Table 5
Evaluation of the α-amylase, α-glucosidase and lipase inhibition activities of ethanolic extracts of Vochysiaceae species.

Samples	α-AMY(%)	α-GLE (%)	LIP (%)
Q. grandiflora LF	92.0 ± 3.5^{a}	14.7 ± 2.7^{a}	NA
Q. grandiflora SB	95.9 ± 1.0^{a}	12.3 ± 0.2^{ab}	NA
V. elliptica LF	59.4 ± 9.1^{b}	6.8 ± 0.8^{c}	1.6 ± 0.4^{c}
V. elliptica SB	97.2 ± 2.8^{a}	3.4 ± 0.5^{c}	35.6 ± 0.3^{a}
Q. parviflora LF	84.8 ± 4.6^{a}	23.7 ± 1.0^{d}	24.8 ± 3.5^{b}
Q. parviflora SB	88.5 ± 4.3^{a}	36.8 ± 3.3^{f}	NA
S. convalliodora LF	57.2 ± 0.1^{bc}	11.1 ± 0.8^{ac}	37.5 ± 0.8^{a}
C. major LF	90.9 ± 3.8^{a}	27.1 ± 0.6^{de}	NA
C. major SB	93.1 ± 3.6^{a}	28.2 ± 1.2^{e}	37.3 ± 1.0^{a}
V. tucanorum LF	44.6 ± 0.3^{cd}	10.2 ± 1.5^{abc}	26.5 ± 0.6^{b}
V. tucanorum SB	33.6 ± 0.3^{d}	13.5 ± 0.4^{a}	NA
V. cinnamomea LF	60.1 ± 7.8^{b}	12.6 ± 1.2^{a}	NA
V. rufa SB	70.9 ± 8.2^{b}	3.5 ± 0.3^{c}	NA
Quercetin	63.5 ± 0.2^{b}	NA	NA
Acarbose	100 ± 0^{a}	71.4 ± 1.8^{g}	NA
Orlistat	–	–	100 ± 0^{d}

Note: Ethanolic extracts of Leaves (LF) and of Stem bark (SB) of Vochysiaceae species. Acarbose was used as a control for α-amylase/α-glucosidase and Orlistat for lipase enzymes. All extracts, quercetin and positive controls were serially diluted in DMSO at a concentration of 10 mg/mL. Values (mean ± standard error) expressed as a percentage of α-amylase (α-AMY), α-glucosidase (α-GLE) and lipase (LIP) inhibition. Different letters indicate significant difference ($p < 0.05$).

assay, the extracts also presented results below 40%, being the extracts of *V. elliptica* SB (35.6 ± 0.3), *S. convalliodora* LF (37.5 ± 0.8) e *C. major* SB (37.3 ± 1.0) those who had the best results. The quercetin was not able to inhibit the α-glucosidase and lipase enzymes, but inhibited the α-amylase by 63.5 ± 0.2.

The Fig. 4 shows the results of IC_{50} values from α-amylase inhibition assay using the ethanol extracts of the Vochysiaceae species, which presented more than 50% of activities. Extracts of LF of *Q. grandiflora*, *V. elliptica* LF, *Q. parviflora*, and *C. major*, showed low IC_{50} results, with values of 5.7 ± 0.6, 5.8 ± 0.5, 4.1 ± 0.5 and 3.2 ± 0.6 μg/mL, respectively. The IC_{50} obtained for SB of *Q. grandiflora*, *Q. parviflora*, *C. major* and quercetin were 3.3 ± 0.7, 6.2 ± 2.0, 11.2 ± 2.8 and 17.5 ± 3.1 μg/mL, respectively. These data shows that the samples presented no significant differences among themselves and with the acarbose (0.4 ± 0.03 μg/mL).

3.4.1. Liquid chromatography-mass spectrometry analysis

The HPLC-ESI-MS/MS analysis was performed to identify the possible bioactive compounds related to biological activities evaluated in this study and their showed in Tables 6 and 7. According to the profiles of fragmentation patterns, error values (ppm) and studies available in the literature, we suggest the presence of several compounds in the extracts of the Vochysiaceae species (Supplementary material S1–S123). The evaluated of mass spectra showed the presence of gliricidin-O-hexoside, myricetin-3-O-(2″-O-galloyl) glucoside, myricetin-3-O-β-D-galactopyranoside, quercetin-glucoside, quinic acid and rutin in *Q. grandiflora* LF and 6-gingerol, ellagic acid rhamnoside, methylellagic acid rhamnoside, quinic acid in *Q. grandiflora* SB.

In extract of *V. elliptica* LF, were identified the (*epi*)catechin, 6-gingerol, isorhamnetin-glucoside, procyanidin b2, quercetin-rhamnoside, rutin and (*epi*)catechin, 6-gingerol, fructose, hederagenin, procyanidin b2, procyanidin c1, quercetin-rhamnoside in SB ethanol extract. In extract of *Q. parviflora* LF, were found the 6-gingerol, kaempferol-glucoside-rhamnoside, quercetin-glucoside, trigalloylglucose and (*epi*)catechin gallate, 6-gingerol, ellagic acid rhamnoside, mallic acid, methylellagic acid rhamnoside, quinic acid in *Q. parviflora* SB extract. In extract of *S. convallariodora* LF, were found (*epi*)catechin, 6-gingerol, coumaroylquinic acid, kaempferol-glucoside, procyanidin B2, quercetin, quercetin-glucoside, quercetin-glucuronide, quinic acid, rutin, sucrose and trigalloylglucose.

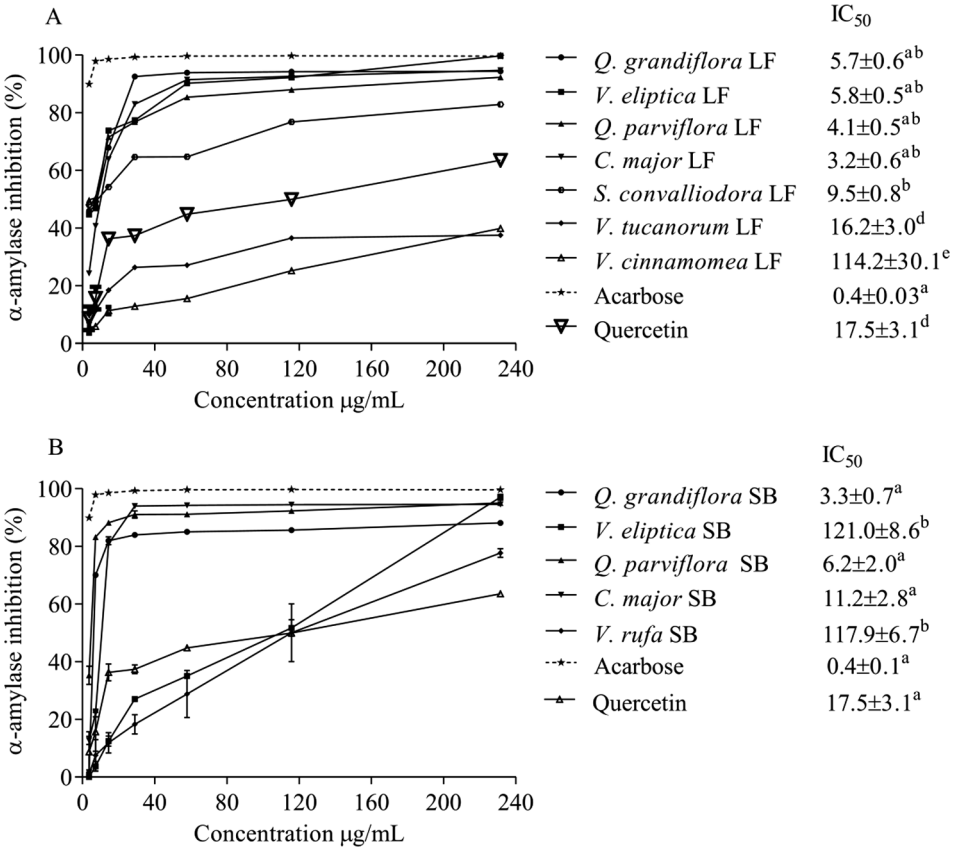


Fig. 4. Half maximal inhibitory concentration (IC_{50}) values of extracts capable of inhibit more than 50% of α-amylase activity. All extracts and positive controls were serially diluted in DMSO at a concentration of 30 mg/mL. Note: (A) Ethanol extracts of Leaves (LF) and (B) Stem bark (SB) of Vochysiaceae species. Acarbose was used as a control for α-amylase/α-glucosidase and Orlistat for lipase enzymes. Different letters indicate significant difference ($p < 0.05$).

Table 6
Compounds identified in the ethanolic extracts of leaves of Vochysiaceae species by HPLC-ESI-MS/MS (negative mode).

Compounds	*Qualea grandiflora* LF	*Vochysia elliptica* LF	*Qualea parviflora* LF	*Salvertia convalliodora* LF	*Callisthene majorLF*	*Vochysia tucanorum* LF	*Vochysia cinnamomea* LF	*m/z* of $[M–H]^-$	*m/z* of fragments of $[M–H]^-$
(*Epi*)catechin	x	✓	x	✓	✓	x	✓	289.0695	245, 221, 203, 165, 137, 125, 109
6-gingerol	x	✓	✓	✓	✓	✓	✓	293.1755	269, 221, 220, 164, 112
Coumaroylquinic acid	x	x	x	✓	x	x	x	337.0910	214, 191, 163, 119
Fructose	x	x	x	x	x	x	✓	179.0533	169, 131, 101
Gliricidin-O-hexoside	✓	x	x	x	x	x	✓	461.1066	417, 299, 229, 178, 127
Hederagenin	x	x	x	x	x	✓	✓	471.3467	347, 311, 267, 234, 152
Isorhamnetin-glucoside	x	✓	x	x	x	x	x	477.1022	433, 357, 315, 271, 186, 150, 136
Kaempferol-glucoside	x	x	x	✓	✓	x	x	447.0916	354, 315, 286, 285, 255, 227, 167
Kaempferol-glucoside-rhamnoside	x	x	✓	x	x	x	✓	593.1487	432, 309, 285, 284, 255, 180, 121
Myricetin-3-O-(2″-O-galloyl) glucoside	✓	x	x	x	x	x	x	631.0939	479, 359, 316, 299
Myricetin-3-O-β-D-galactopyranoside	✓	x	x	x	x	x	x	479.0812	316, 271
Phlorizin	x	x	x	x	x	x	✓	435.1280	359, 257, 193, 167, 137
Procyanidin B2	x	✓	x	✓	x	x	✓	577.1330	451, 407, 289, 243, 199, 162, 125
Quercetin	x	x	x	✓	✓	x	x	301.0344	205, 179, 151, 107
Quercetin-glucoside	✓	x	✓	✓	✓	✓	x	463.0866	300, 255, 151
Quercetin-glucuronide	x	x	x	✓	x	x	x	477.0674	409, 341, 315, 300, 299, 245, 193
Quercetin-rhamnoside	x	✓	x	x	x	✓	✓	447.0925	301, 300, 171
Quinic acid	✓	x	x	✓	x	x	x	191.0523	173, 144, 133, 127, 108
Rutin	✓	✓	x	✓	✓	✓	✓	609.1423	564, 301, 300
Sucrose	x	x	x	✓	✓	✓	x	341.1056	229, 179, 135, 101
Trigalloylglucose	x	x	✓	✓	x	x	x	635.0903	556, 483, 423, 355, 313, 253, 169

Table 7
Compounds identified in the ethanolic extracts of stem barks of Vochysiaceae species by HPLC-ESI-MS/MS (negative mode).

Compounds	*Qualea grandiflora SB*	*Vochysia ellipticaSB*	*Qualea parvifloraSB*	*Callisthene major SB*	*Vochysia tucanorumSB*	*Vochysia rufa SB*	*m/z* of $[M-H]^-$	*m/z* of fragments of [M – H]-
(*Epi*)catechin	x	✓	x	✓	✓	x	289.0695	245, 221, 203, 165, 137, 125, 109
(*Epi*)catechin gallate	x	x	✓	✓	x	x	441.0818	289, 263, 169, 125
(*Epi*)gallocatechin	x	x	x	✓	x	x	305.0658	219, 179, 159, 125, 109
6-gingerol	✓	✓	✓	✓	✓	x	293.1755	269, 221, 220, 164, 112
Ellagic acid rhamnoside	✓	x	✓	✓	x	x	447.0530	300, 209, 169, 150
Fructose	x	✓	x	x	✓	✓	179.0533	169, 131, 101
Hederagenin	x	✓	x	x	x	x	471.3467	347, 311, 267, 234, 152
Mallic acid	x	x	✓	x	x	x	133.0138	115
Methylellagic acid rhamnoside	✓	x	✓	✓	x	x	461.0702	315, 169, 125
Phlorizin	x	x	x	x	✓	x	435.1280	359, 257, 193, 167, 137
Procyanidin B2	x	✓	x	✓	x	x	577.1330	451, 407, 289, 243, 199, 162, 125
Procyanidin C1	x	✓	x	✓	x	x	865.1989	577, 407, 289, 245, 125
Quercetin-rhamnoside	x	✓	x	x	x	x	447.0925	401, 301, 300, 255, 165, 138
Quinic acid	✓	x	✓	✓	x	x	191.0520	171, 137, 127, 109

The *C. major* LF extract presents molecular ions that suggest (*epi*) catechin, 6-gingerol, kaempferol-glucoside, quercetin, quercetin-glucoside, rutin, sucrose and (*epi*)catechin, (*epi*)catechin gallate, (*epi*)gallocatechin, 6-gingerol, ellagic acid rhamnoside, methylellagic acid rhamnoside, procyanidin b2, procyanidin c1, quinic acid in SB extract of this plant. The analysis of *V. tucanorum* LF indicated 6-gingerol, hederagenin, quercetin-glucoside, quercetin-rhamnoside, rutin, sucrose and in *V. tucanorum* SB extract, the compounds found is (*epi*)catechin, 6-gingerol, fructose and phlorizin. In *V. cinnamomea* LF extract were found (*epi*)catechin, 6-gingerol, fructose, gliricidin-O-hexoside, hederagenin, kaempferol-glucoside-rhamnoside, phlorizin, procyanidin B2, quercetin-rhamnoside, rutin and fructose in *V. rufa* SB extract.

4. Discussion

In this work, we carried out a study using LF and SB of selected plants belonging to Vochysiaceae family, evaluating their antioxidant and antiglycation abilities; their inhibitory potential against α-amylase, α-glucosidase and lipase enzymes; their phytochemical constitution by means of colorimetric assays and by liquid chromatography-mass spectrometry analysis. Our main findings indicate that the ethanolic extract of four of eight analyzed plants such as *Q. grandiflora*, *Q. parviflora*, *V. elliptica* and *Callisthene major* exhibited more than 50% of antioxidant and antiglycation capacities and enzymatic inhibitory activities.

Previously, our group and others showed that some medicinal plants have the capacity of inhibit α-amylase, α-glucosidase and lipase, as well as high antioxidant and antiglycation capacities [4,26,29,30]. The antioxidant potential of these plants show the capacity to inhibit the oxidation of cellular molecules [31] and the inhibition of digestive enzymes is related to control of postprandial hyperglycemia and hyperlipidemia [4], which may reduce the inflammations and improve healing process [32]. Besides that, our studies with the aqueous extract of *V. rufa* SB demonstrated that this plant is rich in sugars and has the capacity to reduce hyperglycemia and blood biochemical alterations, besides reducing the oxidative stress in pancreas [17] and liver [15].

Our findings indicate that the ethanolic extracts of the Vochysiaceae species evaluated have a moderate level of reduction molecules, such as polyphenols, flavonoids and condensed tannins. These results can be seen qualitatively by HPLC-ESI-MS/MS analysis (supplementary material), where several polyphenolic compounds were suggested (Tables 6 and 7). According to Nyambe-Silavwe et al. [33], the polyphenols, some suggested here, are described as inhibitors of the human α-amylase enzyme and its isoforms, showing that (*epi*)catechin, quinic acid, gliricidin, hederagenin, isorhamnetin and hamnetin, kaempferol, myricetin, phlorizin, procyanidin B2 and C1, quercetin, rutin, ellagic acid, hederagenin, mallic acid can assist in the inhibition of carbohydrate digestion and in management of postprandial hyperglycemia.

Among the compounds identified, the literature demonstrates that compounds derived from rutin, glucose, fructose, sucrose and ellagic acid were previously found in some species of the Vochysiaceae family [11]. On the other hand, our study suggests the presence of other compounds that have not yet been described for these species, such as quercetin and quercetin glycosides.

Polyphenols, such as quercetin and quercetin glycosides, have a great inhibitory effect of α-amylase, being one of the reasons the large number and position of the OH^- in A, B and C rings presents in their structure [34]. The α-amylase can be strongly inhibited by polyphenols containing galloyl radicals and an unsaturated 2,3-bond in conjugation with a 4-carbonyl group, but methylations, methoxylations and glycosylations in these molecules reduce the polyphenol's ability to inhibit this enzyme [35].

Based on this, some secondary metabolites may be related to the ability of Vochysiaceae species to inhibit the α-amylase enzyme. The extracts LF and SB of *Q. grandiflora*, *V. elliptica*, *C. major* and *Q. parviflora* LF were the samples that stood out in the α-amylase inhibition

assay, since they presented low values of IC_{50} and without significant difference between them and acarbose. In addition to our study, Silva et al. (2009) showed that hydroethanolic extract of *Q. grandiflora* LF inhibited the mammalian and insect α-amylase enzyme.

According to Kim et al. [47], the residues Tyr59 and Gln63 present in the active site of the amylase enzyme interact with the flavonoid quercetin through the π-π interaction and hydrogen bonding due to the hydroxyls present in its A and C rings. In addition, according to Martinez-Gonzalez et al. [48], quercetin interacts with another amino acids Leu165, Trp59 by Van der Waals Interaction and hydrophobic forces and with Trp59, Arg195, Asp197 by hydrogen bonding. Martinez-Gonzalez et al. [48] further states that quercetin apparent decreasing Vmax, indicating a mixed-type inhibition.

The strong inhibitors of α-glucosidase presents valienamine ring and groups such as amides and amines in structure, in addition to iminosugars, carbasugars, thiosugars, and non-sugar derivatives [36]. In addition, the presence of esterified polyphenols containing galloyl radicals are prerequisites for achieving good levels of inhibition of the lipase enzyme, according to Lunagariya et al. [37]. Our results showed that the extracts and quercetin presented low ability to inhibit α-glycosidase and lipase enzymes in the initial assays, with values below 40% of inhibition.

Polyphenols can also stabilize excess free radicals, preventing the establishment of oxidative stress and its harmful action on cellular structures in diseases such as T2DM [38]. Oxidative stress is indicated as the cause or aggravating of more than 50 diseases, such as diabetes mellitus, Alzheimer's, chronic renal failure, arthritis and others [3]. Therefore, most researchers have focused their efforts on evaluating the antioxidant activity and polyphenols content of natural products using some methods [39].

Based on the DPPH, ORAC and FRAP methods, we present results of the potential and antioxidant mechanisms of ethanolic extracts of *V. cinnamomea*, *S. convalliodora* and *C. major*. Still others studies describes the antioxidant capacity of ethanolic extract of *V. elliptica* [19], methanolic extract of *V. tucanorum* [14], aqueous extract of *V. rufa* [16] and methanolic extract of *Q. grandiflora* and *Q. parviflora* stem bark [40]. According to Liang and Kitts [41], the ORAC method evaluates the ability of the extracts in transfer hydrogen atoms (scavenger); FRAP test evaluates the electron transfer mechanism (quenching) and the DPPH, is able to detect the two mechanisms of free radical reduction. The ethanolic extracts of the Vochysiaceae species evaluated in the present work, showed an interesting antioxidant capacity by scavenger and quenching process, probably due to the presence of the identified polyphenols.

There are also many antioxidant polyphenols in natural products that have antiglycation properties and can influence the production of AGEs through different mechanisms, such as inhibition of ROS formation during glycation, inhibition of Schiff base and Amadori products and blocking of AGE–RAGE receptor [42]. In agreement with Wang et al. [28], the BSA/FRU model is used to evaluate the effect of extracts in all stages of protein glycation, while that BSA/MGO and ARG/MGO evaluate, respectively, middle stage and the major amino acid of protein glycation process. Thus, this study brings unpublished information of the antiglycation potential of the ethanol extracts of LF and/or SB of *Q. grandiflora*, *V. elliptica*, *Q. parviflora*, *C. major*, *V. tucanorum*, *S. convallariodora*, *V. cinnamomea* and *V. rufa,* with similar values to quercetin capacity.

The BSA/FRU method is of great importance, since the fructose generated by the oxidation of sorbitol in polyol pathway [43] active, for example, in ocular lenses and peripheral nerves can by accumulate and generate glycated molecules and reactive oxygen species in these tissues, culminating in diabetic's retinopathy [44] and peripheral neuropathy [45], respectively. In BSA/MGO model, the extracts *Q. grandiflora* SB, *V. elliptica* LF and *V. tucanorum* LF showed ability to inhibit the middle glycation stage, but in Arg/MGO, only *C. major* SB and LF showed ability to inhibit glycation of arginine

The methylglyoxal is generated primarily as an intermediate in the metabolism of carbohydrates (glycolysis pathway) [46]; is an important reactive carbonyl in cells and is capable of glycated proteins at a much faster rate than sugars [28]. Inhibition of AGES generated by the reaction with methylglyoxal is extremely important for the treatment of DMT2, because their related to systemic inflammation and other consequences, such as aging, cancers, and vascular complications [46].

In summary, after evaluation of some selected species belonging to the Vochysiaceae family, our results showed that the ethanolic extracts of LF and SB of *Q. grandiflora*, *Q. parviflora*, *V. elliptica* and *Callisthene major* have prominent antioxidant and antiglycation potential, as well as being good inhibitors of the α-amylase enzyme when compared to other species. This study presented new findings about the biological and pharmacological potential of some species from Vochysiaceae family, contributing to the understanding of the action and efficacy of the use of these plants in the digestive enzyme inhibitions and in antiglycation and antioxidant processes. Thus, these results open possibilities for further studies using isolated compounds in order to assess the antidiabetic potential of the aforementioned plants using *in vivo* models.

Declaration of Competing Interest

The authors have declared that there is no conflict of interest.

Acknowledgments

This work was supported by the Foundation for Research Support of the Minas Gerais State (FAPEMIG − APQ-01856- 14 and APQ-02659-12). The phytochemical group from Fundação Ezequiel Dias is grateful to FAPEMIG (DEG-AUC-43/10). National Institute of Science and Technology in Theranostics and Nanobiotechnology—INCT-TeraNano (CNPq/CAPES/FAPEMIG, CNPq-465669/2014- 0 e FAPEMIG-CBB-APQ-03613-17). The authors gratefully acknowledge the Institute of Biotechnology of the Federal University of Uberlândia for infrastructural support. RRF and ABJ received graduate fellowships from Coordination for the Improvement of Higher Education Personnel (CAPES) and FSE is a grant recipient of National Council for Scientific and Technological Development (CNPq).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103122.

References

[1] WHO, World Health Organization. World Health Day 2016: WHO calls for global action to halt rise in and improve care for people with diabetes, World Heal. Org. (2016).
[2] S. Pettit, E. Cresta, K. Winkley, E. Purssell, J. Armes, Glycaemic control in people with type 2 diabetes mellitus during and after cancer treatment: a systematic review and meta-analysis, PLoS ONE 12 (2017) e0176941.
[3] U. Asmat, K. Abad, K. Ismail, Diabetes mellitus and oxidative stress—a concise review, Saudi Pharm. J. 24 (2016) 547–553.
[4] R.R. Franco, D. da Silva Carvalho, F.B.R. de Moura, A.B. Justino, H.C.G. Silva, L.G. Peixoto, F.S. Espindola, Antioxidant and anti-glycation capacities of some medicinal plants and their potential inhibitory against digestive enzymes related to type 2 diabetes mellitus, J. Ethnopharmacol. 215 (2018) 140–146.
[5] N. Gupta, T. Gudipati, G. Prasad, Plant secondary metabolites of pharmacological significance in reference to diabetes mellitus: an update, Int. J. Curr. Microbiol. App. Sci. 7 (2018) 3409–3448.
[6] L.F. de Souza, A família VOCHYSIACEAE A. St.-Hil. na microrregião Sudoeste Goiano-The family VOCHYSIACEAE A. St.-Hil. in the microregion Southwest Goiás, Rev. Biol. Neotrop. 11 (2014) 1–10.
[7] V.E.G. Rodrigues, D.A. de Carvalho, Levantamento etnobotânico de plantas medicinais no domínio do cerrado na região do Alto Rio Grande-Minas Gerais, Ciência e Agrotecnologia. 25 (2001) 102–123.
[8] M.A.B. da Silva, L.V.L. Melo, R.V. Ribeiro, J.P.M. de Souza, J.C.S. Lima, D.T. de, O. Martins, R.M. da Silva, Levantamento etnobotânico de plantas utilizadas como anti-hiperlipidêmicas e anorexígenas pela população de Nova Xavantina-MT, Brasil, Rev. Bras. Farm. 20 (2010) 549–562.

[9] C.A. Hiruma-Lima, L.C. dos Santos, H. Kushima, C.H. Pellizzon, G.G. Silveira, P.C.P. Vasconcelos, W. Vilegas, A.R.M.S. Brito, Qualea grandiflora, a Brazilian "Cerrado" medicinal plant presents an important antiulcer activity, J. Ethnopharmacol. 104 (2006) 207–214.

[10] S.P. Almeida, C.E.B. Proença, S.M. Sano, J.F. Ribeiro, Cerrado: espécies vegetais úteis. Embrapa, Brazil. conservation genetics, Mol. Genet. Approaches Conserv. Oxford Univ. Press. USA. (1998) pp. 54–73.

[11] F.C. Neto, A.C. Pilon, D.H.S. Silva, V. da Silva Bolzani, I. Castro-Gamboa, Vochysiaceae: secondary metabolites, ethnopharmacology and pharmacological potential, Phytochem. Rev. 10 (2011) 413.

[12] L.P. Mazzolin, A.L.M. Nasser, T.M. Moraes, R.C. Santos, C.M. Nishijima, F.V. Santos, E.A. Varanda, T.M. Bauab, L.R.M. da Rocha, L.C. Di, Stasi, Qualea parviflora Mart.: an integrative study to validate the gastroprotective, antidiarrheal, antihemorragic and mutagenic action, J. Ethnopharmacol. 127 (2010) 508–514.

[13] E.M. Silva, A. Valencia, M.F. Grossi-de-Sá, T.L. Rocha, É. Freire, J.E. de Paula, L.S. Espindola, Inhibitory action of Cerrado plants against mammalian and insect α-amylases, Pestic. Biochem. Physiol. 95 (2009) 141–146.

[14] R. De, C. Gomes, F. Bonamin, D.D. Darin, L.N. Seito, L.C. Di Stasi, A.L. Dokkedal, W. Vilegas, A.R. Monteiro Souza Brito, C.A. Hiruma-Lima, Antioxidative action of methanolic extract and buthanolic fraction of Vochysia tucanorum Mart. in the gastroprotection, J. Ethnopharmacol. 121 (2009) 466.

[15] I.B. Moraes, C. Manzan-Martins, N.M. de Gouveia, L.K. Calábria, K.R.N. Hiraki, A. da, S. Moraes, F.S. Espindola, Polyploidy analysis and attenuation of oxidative stress in hepatic tissue of STZ-induced diabetic rats treated with an aqueous extract of Vochysia rufa, Evid.-Based Complement. Altern. Med. 2015 (2015).

[16] N.M. De Gouveia, I.B. Moraes, R.M.F. Sousa, M.B. Neto, A.V. Mundim, A. Oliveira, J.H.G. Lago, F.S. Espindola, Attenuation of oxidative stress in hepatic and pancreatic tissues of STZ-induced diabetic rats treated with aqueous extract of Vochysia rufa, Planta Med. 80 (2014) SL51.

[17] N.M. de Gouveia, W.F. Rodrigues, R.M.F. de Sousa, L.K. Calábria, A.V. Mundim, C.B. Miguel, C.J.F. Oliveira, J.E. Lazo-Chica, A. de Oliveira, J.H.G. Lago, Phytochemical characterization of the Vochysia rufa (Vochysiaceae) extract and its effects on oxidative stress in the pancreata of streptozotocin-induced diabetic rats, PLoS ONE 12 (2017) e0184807.

[18] J.L.C. Lopes, J.N.C. Lopes, H.F. Leitao, Filho, 5-Deoxyflavones from the Vochysiaceae, Phytochemistry 18 (1979) 362.

[19] D.B. da Cruz, Atividade antioxidante e citotoxicidade de plantas do Campo Rupestre brasileiro: Gomphrena arborescens Lf, Gomphrena virgata Mart., Miconia ferruginata DC. e Vochysia elliptica Mart, UFVJM, 2017.

[20] L. Castoldi, T.O. Kelly, L.R. Albiero, E.F. Nery, J.C.S. Dalazen, D.M.S. ValladÃ, Evaluation of the in vitro cytotoxic effects of carvoeiro leaf extracts [Callisthene fasciculata (Spr.) Mart.], Sci. Electron. Arch. 11 (2018) 124–129.

[21] D. de, B. Corrêa, L.F.B. Guerra, A.P. De Pádua, O.R. Gottlieb, Ellagic acids from Callisthene major, Phytochemistry 24 (1985) 1860–1861.

[22] G.A. Lima Neto, S. Kaffashi, W.T. Luiz, W.R. Ferreira, Y.S.A. Dias da Silva, G.V. Pazin, I.M.P. Violante, Quantification of secondary metabolites and antimicrobial and antioxidant activities of some medicinal plants from the Cerrado of the Mato Grosso, Rev. Bras. Plantas Med. 17 (2015) 1069–1077.

[23] M.A.S. Mayworm, M.S. Buckeridge, U.M.L. Marquez, A. Salatino, Nutritional reserves of Vochysiaceae seeds: chemical diversity and potential economic uses, An. Acad. Bras. Cienc. 83 (2011) 523–531.

[24] I.G.C. Bieski, F. Rios Santos, R.M. de Oliveira, M.M. Espinosa, M. Macedo, U.P. Albuquerque, D.T. de Oliveira Martins, Ethnopharmacology of medicinal plants of the pantanal region (Mato Grosso, Brazil), Evid.-Based Complement. Altern. Med. 2012 (2012).

[25] S.M. Paula, E.R. Naves, I.M. Franco, K.J. Manna Padua, K.R. Silva, W.P. Fernandes, D.C. de Oliveira, J.P. de Lemos-Filho, A.S. Franco Pinheiro Moreira, Photosynthetic performance of young and mature leaves of Vochysia cinnamomea (Vochysiaceae) at intact and after fire areas of cerrado rupestre, Biosci. J. 31 (2015) 591–600.

[26] A.B. Justino, N.C. Miranda, R.R. Franco, M.M. Martins, N.M. da Silva, F.S. Espindola, Annona muricata Linn. leaf as a source of antioxidant compounds with in vitro antidiabetic and inhibitory potential against α-amylase, α-glucosidase, lipase, non-enzymatic glycation and lipid peroxidation, Biomed. Pharmacother. 100 (2018) 83–92.

[27] A.B. Justino, M.N. Pereira, D.D. Vilela, L.G. Peixoto, M.M. Martins, R.R. Teixeira, N.C. Miranda, N.M. da Silva, R.M.F. de Sousa, A. de Oliveira, Peel of araticum fruit (Annona crassiflora Mart.) as a source of antioxidant compounds with α-amylase, α-glucosidase and glycation inhibitory activities, Bioorg. Chem. 69 (2016) 167–182.

[28] W. Wang, Y. Yagiz, T.J. Buran, C. do Nascimento Nunes, L. Gu, Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls, Food Res. Int. 44 (2011) 2666–2673.

[29] R.A. Mothana, J.M. Khaled, A.A. El-Gamal, O.M. Noman, A. Kumar, M.F. Alajmi, A.J. Al-Rehaily, M.S. Al-Said, Comparative evaluation of cytotoxic, antimicrobial and antioxidant activities of the crude extracts of three Plectranthus species grown in Saudi Arabia, Saudi Pharm. J. (2018).

[30] A.L. Prada, H. Keita, T.P. de Souza, E.S. Lima, L.D.R. Acho, M. de, J.A. da Silva, J.C.T. Carvalho, J.R.R. Amado, Cassia grandis Lf nanodispersion is a hypoglycemic product with a potent α-glucosidase and pancreatic lipase inhibitor effect, Saudi Pharm. J. (2018).

[31] A.B. Justino, M.N. Pereira, L.G. Peixoto, D.D. Vilela, D.C. Caixeta, A.V. de Souza, R.R. Teixeira, H.C.G. Silva, F.B.R. de Moura, I.B. Moraes, Hepatoprotective properties of a polyphenol-enriched fraction from Annona crassiflora Mart. fruit peel against diabetes-induced oxidative and nitrosative stress, J. Agric. Food Chem. 65 (2017) 4428–4438.

[32] F.B.R. de Moura, A.B. Justino, B.A. Ferreira, F.S. Espindola, F. de Assis Araújo, T.C. Tomiosso, Pro-fibrogenic and anti-inflammatory potential of a polyphenol-enriched fraction from Annona crassiflora in skin repair, Planta Med. (2018).

[33] H. Nyambe-Silavwe, J.A. Villa-Rodriguez, I. Ifie, M. Holmes, E. Aydin, J.M. Jensen, G. Williamson, Inhibition of human α-amylase by dietary polyphenols, J. Funct. Foods. 19 (2015) 723–732.

[34] A.I. Martinez-Gonzalez, Á.G. Díaz-Sánchez, L.A. Rosa, C.L. Vargas-Requena, I. Bustos-Jaimes, Polyphenolic compounds and digestive enzymes: in vitro non-covalent interactions, Molecules 22 (2017) 669.

[35] J. Xiao, X. Ni, G. Kai, X. Chen, A review on structure–activity relationship of dietary polyphenols inhibiting α-amylase, Crit. Rev. Food Sci. Nutr. 53 (2013) 497–506.

[36] E.B. de Melo, A. da Silveira Gomes, I. Carvalho, α-and β-Glucosidase inhibitors: chemical structure and biological activity, Tetrahedron 62 (2006) 10277–10302.

[37] N.A. Lunagariya, N.K. Patel, S.C. Jagtap, K.K. Bhutani, Inhibitors of pancreatic lipase: state of the art and clinical perspectives, EXCLI J. 13 (2014) 897.

[38] Z. Bahadoran, P. Mirmiran, F. Azizi, Dietary polyphenols as potential nutraceuticals in management of diabetes: a review, J. Diab. Metab. Disord. 12 (2013) 43.

[39] B. Dimitrios, Sources of natural phenolic antioxidants, Trends Food Sci. Technol. 17 (2006) 505–512.

[40] C. Bonacorsi, L.M. da Fonseca, M.S.G. Raddi, R.R. Kitagawa, M. Sannomiya, W. Vilegas, Relative antioxidant activity of Brazilian medicinal plants for gastro-intestinal diseases, J. Med. Plants Res. 5 (2011) 4511–4518.

[41] N. Liang, D. Kitts, Antioxidant property of coffee components: assessment of methods that define mechanisms of action, Molecules 19 (2014) 19180–19208.

[42] W.-J. Yeh, S.-M. Hsia, W.-H. Lee, C.-H. Wu, Polyphenols with antiglycation activity and mechanisms of action: a review of recent findings, J. Food Drug Anal. 25 (2017) 84–92.

[43] M. Brownlee, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813.

[44] H.-P. Hammes, X. Du, D. Edelstein, T. Taguchi, T. Matsumura, Q. Ju, J. Lin, A. Bierhaus, P. Nawroth, D. Hannak, Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy, Nat. Med. 9 (2003) 294.

[45] K.E. Schemmel, R.S. Padiyara, J.J. D'Souza, Aldose reductase inhibitors in the treatment of diabetic peripheral neuropathy: a review, J. Diabetes Complic. 24 (2010) 354–360.

[46] I. Allaman, M. Bélanger, P.J. Magistretti, Methylglyoxal, the dark side of glycolysis, Front. Neurosci. 9 (2015) 23.

[47] H.-M. Kim, J.-K. Kim, L.-W. Kang, K.-J. Jeong, S.-H. Jung, Docking and scoring of quercetin and quercetin glycosides against α-amylase receptor, Bull. Korean Chem. Soc. 31 (2) (2010) 461–463.

[48] A. Martinez-Gonzalez, Á. Díaz-Sánchez, L. de la Rosa, I. Bustos-Jaimes, E. Alvarez-Parrilla, Inhibition of α-amylase by flavonoids: Structure activity relationship (SAR), Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 206 (2019) 437–447.