https://dx.doi.org/10.21577/0103-5053.20230070

J. Braz. Chem. Soc., Vol. 34, No. 12, 1773-1787, 2023

Article

Four Brazilian Potential Species Almost Unexplored of Connaraceae Family Used Popularly for Diabetes: Chemical Composition and *in vitro* Activity

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Connaraceae is a plant family usually used in traditional medicine. Recently, we have reported the chemical composition and biological activity of 39 species around the world. This work highlights the application of these species to diabetes control, which has been reported at least to half of this family. In this line, there are several species of the Connaraceae without scientific evidence of traditional use, among these the four species study here. Thus, this work aims the chemical composition prospection of *Connarus blanchetii*, *Connarus regnellii*, *Connarus suberosus* and *Rourea glazioui*, as well as the identification of possible bioactivity. The leaves of these four species were collected, prepared and submitted to different extractive methods (aqueous decoction and infusion and ethanolic maceration), these being tested in a preliminary phytochemical investigation and subjected to antioxidant assay with 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power. Then, we selected the maceration extract to continue for fractions, with the ethyl acetate and *n*-butanol presenting the best results with antioxidant and anti-advanced glycation end products (AGEs) activity potential. Finally, the chemical composition was determinate by high-resolution mass spectrometry (HRMS) in combination with Global Natural Products Service (GNPS) data bank. As a result, this study indicated the presence of 29 phenolics compounds with reported activity as antioxidant and with potential of protein glycation, supported the diabetes activity of this plants. The *n*-butanolic fraction of *R. glazioui* showed compounds such as apigenin, kaempferol, quercetin, myricetin and chlorogenic acid, which have generated a half maximal inhibitory concentration (IC_{50}) of 36.5 μg mL^{-1} for oxidative glycation inhibition, being highlighted as the most active.

Keywords: Connaraceae, antioxidant activity, anti-AGEs activity, chemical composition, GNPS

Introduction

Members of the Connaraceae family are mainly distributed in tropical areas and are comprised of 12 genera and about 200 plant species,[1] 39 of which are associated with pharmacological potential in addition to an ample application in traditional medicine.[2] The use of Connaraceae plants encompasses a wide range of applications in traditional medicine, including the treatment of Diabetes Mellitus (DM), which is reported to some species of the genera *Cnestis* Juss., *Connarus* L. and *Rourea* Aubl.[3-6] In traditional Brazilian medicine species such as *Connarus suberosus* Planch and *Rourea cuspidata* Benth. ex. Baker are used to treat different health problems. The pharmacological

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Editor handled this article: Paulo Cezar Vieira

potential in the diabetes control of *R. cuspidata* reported by traditional communities was confirmed by *in vivo* studies.[6] *Rourea cuspidata* shares with *C. suberosus* at least two compounds, guaijaverin and hyperin, which are associated with beneficial effects on glycemic control.[7] Other Connaraceae species reported around the world with antidiabetic activities comprise preparations obtained from *Cnestis ferruginea* DC.,[3] *Rourea coccinea* (Schumach. & Thonn.) Benth.[4,8] and *Rourea minor* (Gaertn.) Alston.[5,9] Some of these species were tested and proven to be active in controlling blood glucose in rats whose diabetes was induced by streptozotocin or alloxan.[3,4,6] Reviewing the potential of the chemical composition of the Connaraceae species, we found that several flavonoids have glycation inhibitory activity and antioxidant potential.[2,7] Thus, we believe that the beneficial effects of Connaraceae metabolites in controlling DM may not only be associated with the hypoglycemic effect as reported in the literature for several species[4-6] but may extend to the inhibition of protein glycation (IAPG) activity already reported for the *C. ferruginea*[10] and the reduction of oxidative stress as demonstrated in *R. coccinea.*[4]

Diabetes Mellitus is a persistent disorder caused by elevated blood glucose that affects the metabolism of carbohydrates, lipids and proteins.[11] Hyperglycemia is the factor that triggers long-term complications, causing oxidative damage followed by difference between the production of reactive oxygen species (ROS) or the antioxidant defense mechanisms.[12-14] Evidence suggests that diabetic patients are more exposed to oxidative stress because they have a higher production of ROS than patients who do not have the disease.[14] In diabetes, mitochondrial processes in oxidative phosphorylation represent the main source of free radicals contributing to non-enzymatic glycation of proteins, glucose oxidation, increased lipid peroxidation, damage to enzymes and increased insulin resistance.[12] Evidence suggests that even patients treated with oral hypoglycemic drugs are susceptible to oxidative stress since these drugs cannot reverse all of the changes caused by hyperglycemia.[15] Clinical studies[13] have shown that antioxidant treatments with vitamins C and E, and α-lipoic acid offered positive results in the prevention of complications from diabetes. Among the many pathophysiological changes resulting from DM, the accelerated generation of progress advanced glycation end products (AGEs) associated with chronic hyperglycemia leads to the cell and tissue damage observed in the progression of DM.[16,17] AGEs are a heterogeneous group of products that are permanently formed through non-enzymatic glycation and oxidation of proteins, nucleic acids and lipids, which can promote cell death and contribute to the advance of diabetic complications,[18] including nephropathy, neuropathy and retinopathy.[17]

Researchers have shown that phenolic compounds, mainly flavonoids, are active against the inhibition of AGE formation.[14,17,19] Flavonoids are widely reported as metabolites in Connaraceae.[6,20-22] In this context, considering the pharmacological potential of this family of plants, this work aims to analyze the chemical composition and the antioxidant and anti-AGEs potential of four species of Connaraceae: *Connarus blanchetii* Planch.; *Connarus regnellii* G. Schellenb.; *Connarus suberosus* Planch and *Rourea glazioui* G. Schellenb. In the first step, all species were subjected to ethanolic maceration, followed by extraction with different solvents, where the metabolites were quantified. Sequentially, the antioxidant and anti-AGEs activities were evaluated from the richest fractions, and the chemical compounds were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and identified via the Global Natural Product Social Molecular Networking site (GNPS), complemented by other bioinformatics platforms.

Experimental

Reagents

Catechin, gallic acid, quercetin, vanillin sodium acetate ($C_2H_3NaO_2$), 2,4,6-tripyridyl-*s*-triazine (TPTZ), ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), glyoxal, phosphate buffered saline (PBS), bovine serum albumin (BSA), sodium azide, aminguanidine, and fructose were purchased from Sigma-Aldrich (Saint-Louis, Missouri, USA). Folin-Ciocalteu reagent, aluminun chloride ($AlCl_3$), and petroleum ether were supplied by Êxodo (Sumaré, SP, Brazil). Sodium carbonate (Na_2CO_3) was purchased from Synth (Diadema, SP, Brasil). Dimethyl sulfoxide (DMSO) was purchased from Tedia (Fairfield, OH, USA). Sodium nitrate ($NaNO_3$), hexane, dichloromethane, ethyl acetate and *n*-butanol were bought from Dinâmica (São Paulo, SP, Brazil). Ethanol, methanol, hydrochloric acid (HCl), 2,2-diphenyl-1-picrylhydrazyl (DPPH), glacial acetic and formic acid were supplied by Merck (São Paulo, SP, Brazil). All chemicals were of analytic grade.

Plant material

The plants access was registered at the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen).[23] Table 1 shows details about the plants. These were individually dehydrated in a greenhouse with dry air flow at a controlled

Table 1. Plant material of Brazilian species (Connaraceae) used in this work

Species	Acronym	Location City (State)	Biome	Coordinates	Voucher (Herbarium)	SisGen No.
C. blanchetii	CBL	Ilhéus (BA)	Atlantic Forest	14°55'56" S, 39°1'32" W	C. Toledo 404 (ESA 143612)	A1FE9E7
C. regnellii	CRL	Piedade (SP)	Atlantic Forest	23°42'05" S, 47°30'38" W	C. Toledo 377 (ESA 143608)	A7E69F6
C. suberosus	CSL	Brasília (DF)	Cerrado	15°56'51" S, 47°51'58" W	J. Paz 91 (UB Paz 91)	A251027
R. glazioui	RGL	Conceição da Barra (ES)	Atlantic Forest	18°27'49" S, 39°43'28" W	C. Toledo 400 (ESA 143609)	A1FE9E7

CBL: *C. blanchetii* leaves; CRL: *C. regnellii* leaves; CSL: *C. suberosus* leaves; RGL: *R. glazioui*; ESA: Escola Superior de Agricultura "Luiz de Queiroz"; UB: Universidade de Brasília.

temperature of 35 °C for 7 days, and subsequently, they were ground in a knife mill, Willye Model TE 650 Tecnal® (Piracicaba, SP, Brazil).

Extraction

Decoction (D)

The decoction method was conducted following Oliveira *et al*.,[24] with some modifications: 20 g of the obtained powder was added to water preheated at 100 °C (100 mL) and maintained at a constant temperature of 100 °C in heating plates for 30 min, under continual agitation. This procedure was repeated twice, and the combined supernatants were decanted and centrifuged (3000 × g for 5 min at 20 °C), filtered (12-25 µm), solvent removed by rotative evaporation (Rotavapor® Buchi R210) and lyophilized for 24 h in a freeze drier (Labconco Freezone® 4.5 Plus, Barcelona, Spain).

Infusion (I)

This method was conducted following Kalegari *et al*.,[21] with some modifications: 20 g of the obtained powder was infused with water preheated at 70 °C (100 mL) for 30 min under continual agitation at room temperature. This procedure was repeated twice, and the combined supernatants were decanted and centrifuged (3000 × g for 5 min at 20 °C), filtered (12-25 µm), solvent was removed by rotative evaporation and the recovery process was conducted according to decoction method.

Maceration (tincture) (M)

This method was conducted according to literature,[25] with some modifications: 20 g of the obtained powder was macerated twice in ethanol (100 mL) for 24 h under continual agitation at room temperature. After decantation and centrifugation (3000 × g for 5 min at 20 °C), the recovered and combined supernatants were filtered (12-25 µm) and then solvent was removed by rotative evaporation.

Determination of phenolic content

Total phenolic content of *C. blanchetii* (CBL), *C. regnellii* (CRL), *C. suberosus* (CSL) and *R. glazioui* (RGL) extracts was determined by Folin-Ciocalteu method,[26] with minor modifications. Briefly, 100 µL of the extracts (1 mg mL^{-1} in distilled water) was added to 7.4 mL of distilled water and 500 µL of Folin-Ciocalteu reagent. After 1 min of equilibration, the mixture was neutralized with 2 mL of 15% (m/m) Na_2CO_3. After 30 min of reaction, the absorbance of the mixture was measured at 750 nm in a UV-Vis spectrophotometer (Beckmann DU 530, Hudson, USA). Gallic acid (7.81-500 µg mL^{-1}) was used as a standard, and the total flavonoid content was calculated using the calibration curve for gallic acid. Amounts of phenolics were calculated from a gallic acid standard curve and expressed as µg of gallic acid equivalent *per* mg of dry extract.

Determination of flavonoids contents

Flavonoid contents of CBL, CRL, CSL and RGL extracts were valued according to the method described by Gomes *et al*.,[26] based on aluminum chloride reaction with extract. To 1 mL of the extract (1 mg mL^{-1} in methanol) was added 4 mL of distilled water and 200 µL of 5% (m/m) $NaNO_3$. After 6 min, 200 µL of 10% (m/m) $AlCl_3$ were added, and the mixture rested for 5 min. Then, 2 mL of 10% (m/m) NaOH was added, and the total volume was brought to 10 mL with methanol. The absorbance was evaluated in a UV-Vis spectrophotometer (Beckmann DU 530) after 30 min at 425 nm. Quercetin (7.81-500 µg mL^{-1}) was used as a standard, and the total flavonoid content was calculated using the calibration curve for quercetin. The absorbance of the obtained yellow complex was measured at 430 nm. The total of flavonoids was calculated from a quercetin standard curve and expressed as µg quercetin equivalent *per* mg of dry extract.

Determination of condensed tannins content

Condensed tannins contents of CBL, CRL, CSL and RGL extracts were estimated according to the method described by Janovik *et al.*[27] In this reaction, the condensed tannins are converted to generate anthocyanidins.[28] Briefly, 100 µL of extract solution (250 µg mL^{-1} in methanol) were mixed with 2.5 mL of vanillin solution at 1% in methanol (m/v) and 2.5 mL of solution HCl 8% in methanol (v/v). After 15 min, the solution was disposed in a water bath previously heated to 60 °C for 10 min and the absorbance was measured in a UV-Vis spectrophotometer (Beckmann DU 530) at 500 nm. Catechin (62.5-1.000 µg mL^{-1}) was used as a standard, and the condensed tannins content was calculated using the calibration curve for catechin. Amounts of condensed tannins were calculated from a catechin standard curve and expressed as µg catechin equivalent *per* mg of dry extract.

Extraction and partition maceration (tincture) - M

As previously presented here, for three cycles, 80 g of obtained powders were macerated twice in ethanol (400 mL) for 24 h under continual agitation at room temperature. After decantation and centrifugation (3000 × g for 5 min at 20 °C), the recovered and combined supernatants were filtered (12-25 µm) and then the solvent was removed by rotative evaporation (Rotavapor® Buchi R210). The dry extract was resuspended in 100 mL H_2O/ethanol 8:2 (v/v). Sequentially, the extract resuspended was degreased with petroleum ether in separatory funnel and extracted at room temperature with hexane, dichloromethane, ethyl acetate and *n*-butanol (3 cycles with 100 mL for each solvent).[29] Figure 1 showed the process summarized.

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) - screening

A screening of the percentage of DPPH• scavenging of CBL, CRL, CSL and RGL extracts (125 µg mL^{-1} in methanol) was valued according to the method described previously[30] with minor modifications. Briefly, 0.3 mL of each extract was added to 2.7 mL of DPPH• at a concentration of 40 µg mL^{-1} and the mixture was kept protected from light for 30 min. After, the absorbance was measured in a UV-Vis spectrophotometer (Beckmann DU 530) at 515 nm. A mixture of methanol (2.7 mL) and methanolic extract solution (0.3 mL) was used as blank. The negative control was a solution of methanol (0.3 mL) and DPPH• (2.7 mL). Tests were performed in triplicate, and DPPH• scavenging (SC / %) activity was calculated as follows:

$$SC\ (\%) = [(A_{Neg.control} - A_{sample})/A_{Neg.control}] \times 100 \quad (1)$$

where, $A_{Neg.control}$ and A_{sample} are the average absorbance values of the negative control and samples, respectively.

Determination of scavenging concentration

The scavenging concentration (SC_{50}) of the ethyl acetate and *n*-bunanolic fractions was established using serial dilutions of the dry extract (15.6 to 62.5 µg mL^{-1}) following the same method previously presented.

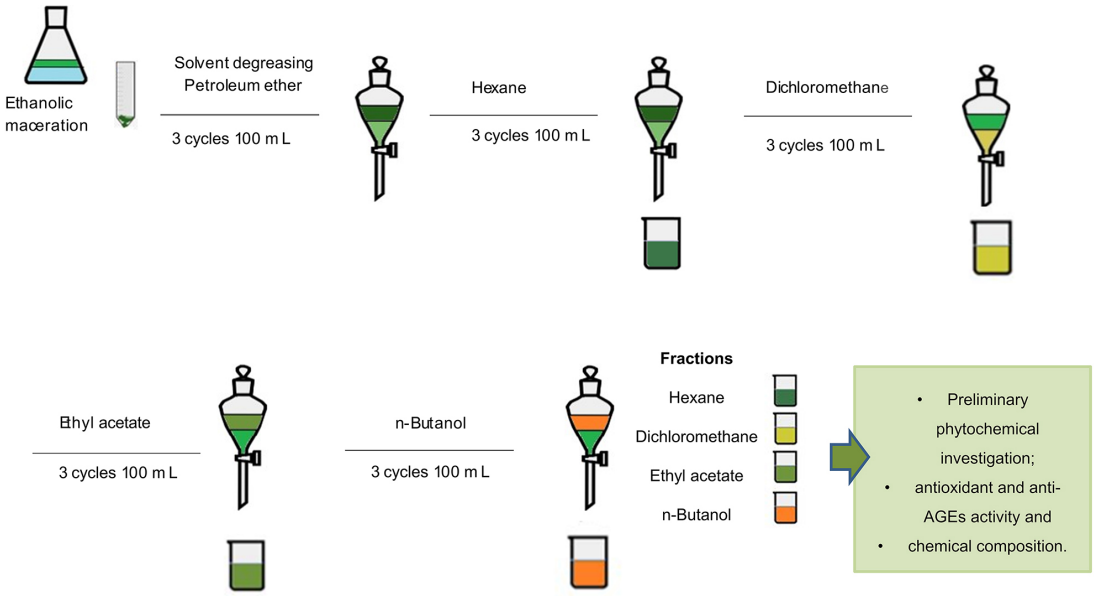

Figure 1. Schematic representation of the extraction process through maceration.

Ferric reducing antioxidant power (FRAP) assay

The FRAP of the ethyl acetate and *n*-bunanolic fractions was determined assay was performed according to the method used by Thaipong *et al*.,[31] with modifications. Stock solutions were composed from: acetate buffer 300 mM, pH 3.6 (3.1 g of sodium acetate, 16 mL of glacial acetic acid with the volume completed up to 1 L with deionized water); 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution 10 mM (3.12 g of TPTZ dissolved in a 40 mM HCl aqueous solution with the volume made up to 1 L); ferric chloride solution ($FeCl_3 \cdot 6H_2O$) 20 mM (5.4 g of $FeCl_3 \cdot 6H_2O$ dissolved in deionized water up to 1 L). The FRAP solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37 °C before use. Connaraceae ethanolic extracts 16.2 to 250 μg mL^{-1} (90 μL), deionized water (270 μL) was allowed to react with 2850 μL of the FRAP solution for 30 min in the dark condition. The colored product (ferrous tripyridyltriazine complex) was then measured at 595 nm in a UV-Vis spectrophotometer (Beckmann DU 530). The standard curve was linear between 500 to 2.000 μM using ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was performed according to Pulido *et al*.[32] The results were expressed in μg ext dry mL^{-1} eq. 1.000 mM ($FeSO_4.7H_2O$).

Inhibitory effect in advanced glycation endproducts (AGEs)

Bovine serum albumin and glyoxal model (BSA/GO)

The method of measuring anti-AGE activity via the oxidative pathway,[33] was prepared in accordance to the literature with some modifications.[34] The dry extracts were prepared in dimethyl sulfoxide (DMSO) (100 μg mL^{-1}). The glyoxal (30 mM) and BSA (bovine serum albumin) (10 mg mL^{-1}) solution was prepared in phosphate buffer (0.2 M, pH 7.4) containing 3.0 mM sodium azide as an antimicrobial agent. The reactions were performed with 300.0 μL of the total reaction mixture composed by (BSA (135.0 μL), glyoxal (135.0 μL) and DMSO or sample (30.0 μL)), and incubated at 37 °C. After 48 h of incubation, the sample was analyzed for fluorescence intensity using a microplate reader (excitation at 330 nm and emission at 420 nm) (DTX 800, Beckman Coulter, CA, USA). DMSO was used as the negative control, and quercetin (100.0 μg mL^{-1}) was used as the standard. The experiment was performed in triplicate. The percentage of inhibition was calculated using equation 2:

$$\% \text{ inhibition} = 100 - (A_2 \text{ sample} - A_1 \text{ sample}/A_2 \text{ control} - A_1 \text{ control}) \times 100 \quad (2)$$

where A_1 is the fluorescence of the initial reading and A_2 is the fluorescence of the final reading.

For all extracts at 100 μg mL^{-1} whose inhibition percentage was greater than 50%, the respective half maximal inhibitory concentration (IC_{50}) was determined using serial dilutions of the dry extract (10-100.0 μg mL^{-1}) in DMSO.

Bovine serum albumin and fructose model (BSA/fructose)

Anti-AGE activity, measured using the non-oxidative pathway method, was determined according to the method described by Kiho *et al*.[33] with some modifications.[34] Utilizing the same methodology as described for bovine serum albumin and glyoxal model (BSA/GO), the incubation time was set at 72 h and used fructose (0.10 mM) instead of glyoxal. Aminoguanidine was used as the standard. The assay was performed in triplicate. The IC_{50} was determined using serial dilutions of the dry extract (6.0-100.0 μg mL^{-1}) in DMSO.

Chemical composition

LC-MS/MS analysis

The LC-MS analysis was performed as described by Paim *et al*.,[7] in Shimadzu 20A series HPLC system with binary solvent delivery, degas system, auto sampler and SPD-20A UV-Visible detector (dual channel λ 254 and 320 nm). Separation method was performed with an octadecylsilyl C_{18} analytical column (4.6 × 250 mm), with particles of 5 μm. The mobile phase was in gradient mode: A - water/formic acid 0.1% v/v; B - and methanol/formic acid 0.1% (v:v), which were eluted at 1 mL min^{-1} as follows: 13.8% of B at 0-45 min; 28% of B at 45-60 min; 100% of B at 60-80 min and finally with 13.8% of B at 80-82 min. Mass spectrometric analysis was performed using Bruker® MicroTof-QII spectrometer with electrospray ionization source (ESI) operated in positive ionization mode. ESI source was operated at 200 °C with an ionization voltage of 35-40 eV and sheath gas flow rate of 8 L min^{-1}. The analysis was performed at *m/z* range of 100-1200 and a normalized collision energy of 10 eV at 15000 resolution full width at half maximum (FWHM) was used for the survey scans.

Data analysis-molecular network

A molecular network was generated using the online workflow on the GNPS website.[35] The spectra were window cleaned by choosing only top 6 fragment ions in the ± 50 Da window throughout the spectrum. The precursor ion mass acceptance was set to 0.02 Da, with a MS/MS fragment ion tolerance of 0.02 Da.[36] A network was then formed in

which edges were filtered to have a cosine score above 0.7 and more than 6 matched peaks. Additionally, edges between two nodes were only kept in the network if each of the nodes showed in each other's respective top 10 most similar nodes. At last, the maximum size of a molecular family was set to 100, and the lowest scoring edges were removed from molecular families until the molecular family size was below this threshold. The spectra in the network were then searched against GNPS spectral libraries. The library spectra were filtered in the same manner as the input data. All matches between network spectra and library spectra were required to have a score above 0.7 and at least 6 matched peaks. The results were downloaded and posted to be visualized on Cytoscape 3.8.2 software.[37]

Complementary analysis-CFM-ID and ChemCalc plataforms

To complement and check the identification made by the GNPS platform, in addition to the retention times of the metabolites present in the extracts, tool spectral prediction was used.[38] This tool provides low energy/10 V, medium energy/20 V and high energy/40 V MS/MS spectra for a required input structure in the Simplified Molecular Input Line Entry System (SMILES) format. Spectra of compounds are produced using combinatorial fragmentation.[39,40] The SMILES of the compounds were carried-out from website,[41] and the data were then submitted to the work tool flows in the following parameters: spectra type: ESI; ion mode: positive; adduct type: $[M + H]^+$ spectra peaks and possible matching fragments for the compounds were evaluated in 40 V, a similar energy to that used in the LC-MS analysis. Additionally, all matching fragments had their chemical formulas searched in the MF Finder tool on the ChemCalc platform.[42]

Results and Discussion

Different extractive methods and preliminary analysis

The use of medicinal plants has increased around the world with the dissemination of ethnopharmacological knowledge and the addition of related scientific information, as well as cultural issues in specific regions of the planet.[43-45] In this sense, plants have usually been used in the form of infusions, decoctions and macerations,[24] with the literature reporting that the same forms are used for species of the Connaraceae family: decoction,[46-48] infusion[49] and maceration.[50]

Thus, in order to recognize the most effective process for the production of phenolic compounds, as a first step, we have determined the total polyphenol and flavonoid content of preparations carried out by decoction, infusion and maceration. Additionally, (2,2-diphenyl-1-picrylhydrazyl radical) DPPH$^{\bullet}$ scavenging activity screening was conducted. The results are reported in Figures 2a-2c.

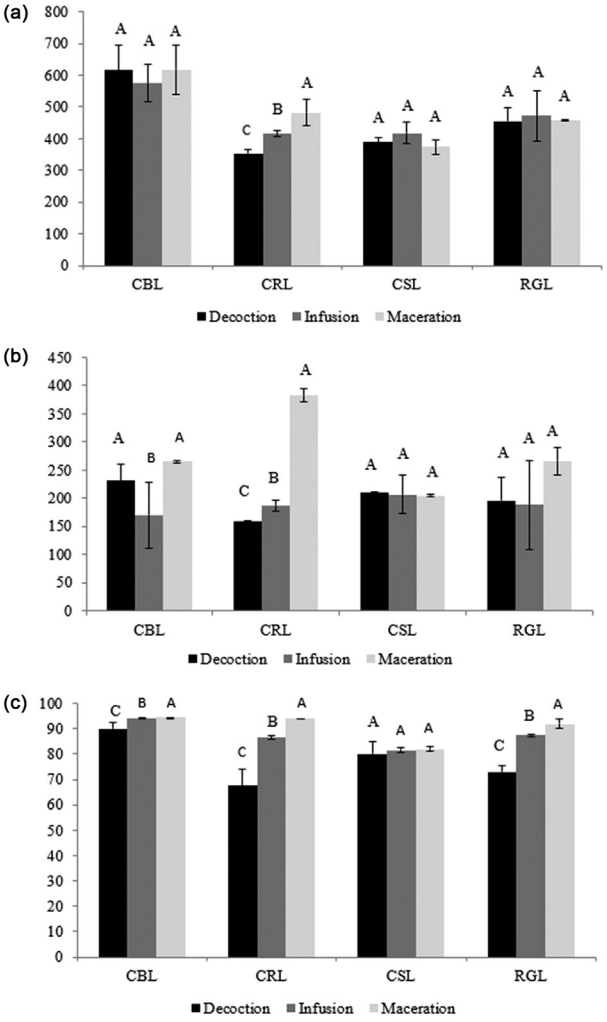

Figure 2. Results for the preliminary analysis. (a) Total polyphenol content expressed as μg of gallic acid equivalent *per* mg of dry extract, (b) flavonoid content expressed as μg of quercetin equivalent *per* mg of dry extract and (c) (2,2-diphenyl-1-picrylhydrazyl radical) DPPH$^{\bullet}$ scavenging % (SC%) activity, where CBL = *C. blanchetii*, CRL = *C. regnellii*, CSL = *C. suberosus* and RGL = *R. glazioui.*

Analysis of the total polyphenol contents, Figure 2a, shows that maceration had a better quantitative profile for *C. regnellii* leaves (CRL), while this method did not produce significant changes for the other species. For the total flavonoids, Figure 2b, maceration was the method with the best performance for *C. blanchetii* leaves (CBL) and CRL. In the DPPH$^{\bullet}$ screening, Figure 2c, maceration presented the highest percentage of radical scavenging for CBL, CRL and *R. glazioui* leaves (RGL). In our previous article,[7] we reported some changes in the qualitative

profile of phenolic compounds for four species of the genus *Connarus*. However, from the quantitative point of view, as far as we know, there is no work reporting on the comparison between methods of extraction. Similar works have shown differences in the phenolic profile in accordance with the extraction method for species such as *Dicksonia sellowiana* Hook. and *Syzygium cumini* (L.) Skeels. For *D. sellowiana*, the polyphenols, flavonoids and protoanthocyanins extracted by decoction, infusion and maceration showed different quantitative profiles, which were associated with changes in the antioxidant potential measured by DPPH•.[24] In *S. cumini*, different extractive processes, including infusion and ethanolic maceration, produced changes in the quantitative profile of total polyphenols and activity against DPPH•.[51] Other authors[24,25,52] have reported that phenolic yields depend on different factors, including the type of solvent used, the plant matrix and the duration of the extractive process employed. In this way, from these results, we have selected maceration as an extractive method for the next steps of this work.

Organic fractions and new quantitative analysis

In the sequence, we have evaluated the polyphenol content from the selected method with different organic solvents (Figure 3). In this stage, the maceration extract was sequentially fractioned using hexane, dichloromethane, ethyl acetate and *n*-butanol, and were then analyzed again for phenolic, flavonoid and tannin content, in addition to the DPPH• screening. From these results, we highlighted the extracts carried out with polar solvents. In the analysis of the scavenging (SC%) with DPPH• (Figure 3d), the elimination percentage was higher for the ethyl acetate and *n*-butanol fractions. In the quantification of total flavonoids (Figure 3b), the ethyl acetate fraction for CBL, CRL and *C. suberosus* leaves (CSL), and the dichloromethane fraction for RGL were respectively highlighted, which is in accordance with that reported for other Connaraceae species.[28,53-55] Thus, we used the ethyl acetate and *n*-butanol fractions in the next step.

In vitro analysis-antioxidant potential

Evaluation of the antioxidant potential of the ethyl acetate and *n*-butanol fractions was studied using the DPPH• experiments, where the respective SC50 were determined, measured in μg mL^{-1} of dry extract (μg ext dry mL^{-1}) (Table 2). In addition, a ferric reducing antioxidant power (FRAP) assay was conducted, with results expressed as μg mL^{-1} of dry extract equivalent to 1000 mM of ferrous sulfate ($FeSO_4.7H_2O$).

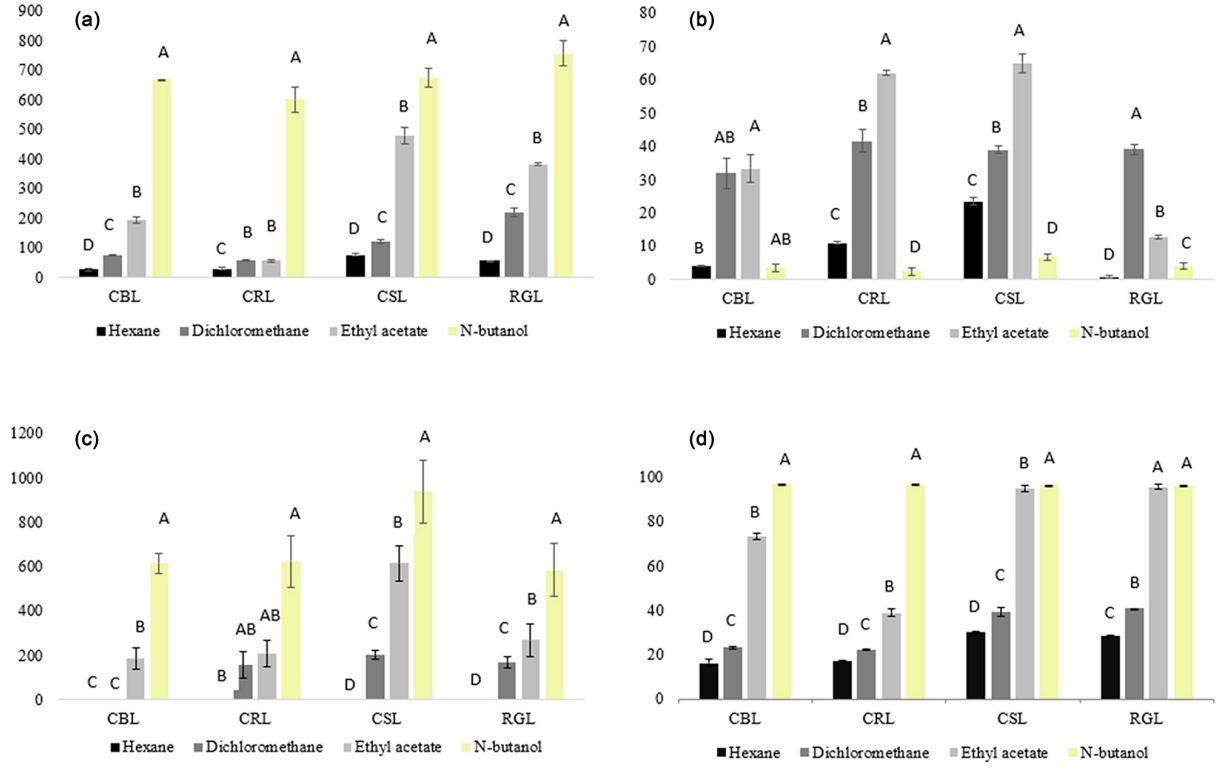

Figure 3. Results for organic fractions. (a) Total polyphenol content expressed as μg of gallic acid equivalent *per* mg of dry extract, (b) flavonoid content expressed as μg of quercetin equivalent *per* mg of dry extract, (c) condensed tannins content expressed as μg catechin equivalent *per* mg of dry extract and (d) (2,2-diphenyl-1-picrylhydrazyl radical) DPPH• scavenging % (SC%) activity, where CBL = *C. blanchetii*, CRL = *C. regnellii*, CSL = *C. suberosus* and RGL = *R. glazioui.*

In the DPPH• assay, the *n*-butanol fraction showed a better performance for all species, in comparison with the ethyl acetate fraction. In the FRAP assay, CBL, CRL and CSL showed the best iron-reducing power, while the ethyl acetate fraction showed the best results for RGL. Antioxidant assays show different reaction behavior against the chemical composition, as well as the solubility of molecules in different solvents.[56] Thus, these methods have some limitations and show different tendencies to react against different classes of phenolic compounds.[57] In the same way, a correlation between the level of these compounds and the antioxidant activity is reported for several species, including *Lagenaria siceraria* (Molina) Standl.,[53] *Anthemis praecox* Link,[54] *Ononis mitissima* L.[55] *Allophylus edulis* (A.St.-Hil., A.Juss. & Cambess.) Radlk. and *Cupania vernalis* Cambess., where the ethyl acetate and *n*-butanol fractions have shown the most promising results.[58] Combining all the results, we chose to analyze the antiglycation potential of the *n*-butanol fraction for all species, in addition to the ethyl acetate fraction of *R. glazioui.*

Advanced glycation end products (AGEs) inhibitory effects

The IAPG activity was evaluated for the oxidative and non-oxidative pathways (Table 3) in order to assess the ability of extracts to inhibit protein glycation in the presence of an oxidizing agent (glyoxal) and in its absence, respectively.

The extracts CBLnb, CSLnb (*n*-butanol) and RGLea (ethyl acetate) were tested for IAPG at a concentration of 100 μg mL^{-1} and showed values greater than 50% inhibition, from which the IC_{50} values were determined. The best result was seen for RGLea, with an IC_{50} of 36.5 μg mL^{-1}, which was better than that observed for the quercetin standard. In the assessment of the inhibitory activity for the non-oxidative pathway, all extracts showed an inhibition percentage greater than 50% when tested at a concentration of 100 μg mL^{-1}. In this case, all IC_{50} values were determined, and RGLea was found to have the most potent effect at 4.5 μg mL^{-1}, which was lower than that observed for the standards quercetin 21.2 μg mL^{-1} and aminoguanidine 36.3 μg mL^{-1}. For CBL, CRL, CSL and RGL, the IC_{50} values were 8.4, 9.4, 22.1 and 13.7 μg mL^{-1}, respectively.

The search for chemical compounds that can inhibit protein glycation implies benefits to diabetic patients. Some plant species have demonstrated their anti-AGEs effects, such as *Ilex paraguariensis* A St.-Hil.,[19] *Eugenia punicifolia* (Kunth) DC.[59] and *Myrcia multiflora* (Lam.) DC.[34] In Connaraceae, for the *in vitro* model of the species *C. ferruginea*, the methanol

Table 2. *In vitro* antioxidant activity via DPPH• and FRAP assays for the ethyl acetate and *n*-butanol fractions

entry	(DPPH•) radical scavenging		Ferric reducing antioxidant power (FRAP)	
	Ethyl acetate	*n*-Butanol	Ethyl acetate	*n*-Butanol
	SC_{50} / (μg ext dry mL^{-1})	SC_{50} / (μg ext dry mL^{-1})	(μg ext dry mL^{-1} eq. 1.000 mM $FeSO_4 \cdot 7H_2O$)	
CBL	146.6	38.5	277.4	75.6
CRL	601.8	40.2	1477.0	81.3
CSL	80.7	31.1	183.7	72.5
RGL	92.4	39.4	78.9	143.2

CBL: *C. blanchetii*; CRL: *C. regnellii*; CSL: *C. suberosus*; RGL: *R. glazioui.*

Table 3. Anti-AGE activities of the dry fractional extracts of the Connaraceae leaves

Sample	Oxidative glycation inhibition		Non-oxidative glycation inhibition	
	Inhibitory effect at 100 μg mL^{-1} / %	IC_{50} / (μg mL^{-1})	Inhibitory effect at 100 μg mL^{-1} / %	IC_{50} / (μg mL^{-1})
CBLnb	54.0 ± 1.2	78.0 (71.6 to 84.9)	96.5 ± 1.5	8.4 (5.5 to 12.7)
CRLnb	47.3 ± 1.0	NT	95.5 ± 0.5	9.4 (6.2 to 14.2)
CSLnb	58.5 ± 0.7	54.9 (50.2 to 60.0)	96.7 ± 0.9	22.1 (13.4 to 36.3)
RGLea	77.6 ± 1.2	36.5 (34.0 to 39.3)	93.7 ± 1.2	4.5 (3.0 to 6.8)
RGLnb	49.1 ± 2.2	NT	98.5 ± 0.7	13.7 (7.6 to 24.8)
Naringenin	33.3 ± 5.0	NT	4.6 ± 0.6	NT
Quercetin	58.4 ± 1.0	46.3 (37.4 to 57.3)	96.0 ± 0.1	21.2 (12.8 to 35.2)
Acarbose	NT	NT		NT
Aminoguanidine	NT	NT	77.4 ± 2.2	36.3 (29.8 to 44.3)

CBL: *C. blanchetii*; CRL: *C. regnellii*; CSL: *C. suberosus*; RGL: *R. glazioui*; nb: *n*-butanol; ea: ethyl acetate. NT: not tested; IC_{50}: half maximal inhibitory concentration.

extraction of the leaves at a concentration of 30 μg mL^{-1} was shown to reduce the glycation of human red blood cells by 80%, a result similar to the effect shown by the flavonoid quercetin, which was tested at the same concentration.[10]

Chemical composition

In order to establish the profile of chemical components that are involved with the biological effects, we studied the *n*-butanol fraction of all species, in addition to the ethyl acetate fraction of RGL. The data obtained by LC-MS/MS analysis were analyzed using the molecular networking tool of the GNPS platform. Sequentially, the identification was complemented by competitive fragmentation analysis, where the fragment ions compatible with metabolites were proposed via spectral prediction (SP) using Competitive Fragmentation Modeling for Metabolite Identification CFM-ID.[39,40] In the last step, the respective molecular formulas of the fragmentation ions that were compatible between the fragmentation produced in the MS/MS analysis and the SP were confirmed in ChemCalc.[60] Figure 4 demonstrates the molecular network with all compounds identified.

By combining these tools, it was possible to propose the molecular network with the identification of 29 compounds. Among them, the flavanols kaempferol $[M + H]^+$ *m/z* 287.0550 (compound **2**), quercetin $[M + H]^+$ *m/z* 303.0500 (compound **5**) and myricetin $[M + H]^+$ *m/z* 319.0448 (compound **8**) form a single cluster with three nodes, separated by a mass variation of 16 Daltons (Da), which is compatible with the mass of an oxygen atom among the chemical formulas of these metabolites, as confirmed using MF finder from ChemCalc. Kaempferol and quercetin are present in *C. regnellii*, *C. suberosus* and *R. glazioui*, whereas myricetin has been identified in *C. blanchetii*, *C. suberosus* and *R. glazioui*. In our previous article,[2] we described kaempferol, quercetin and myricetin in crude extracts obtained from four taxa of the genus *Connarus*. The fragmentation obtained for kaempferol, quercetin and myricetin can be visualized in Figures S2, S5 and S8 (Supplementary Information section), where the measured and predicted fragments are identified, together with the chemical formulas to which they refer.

In the cluster referring to glycosylated flavonoids, 11 nodes represent heterosides for these species, with 10 being identified. Quercetin-3-*O*-pentoside (compound **15**), ion $[M + H]^+$ *m/z* 435.0900, is one of the metabolites associated with CSLnb and RGLea, being a glycosylated flavonoid derived from quercetin, with genin fragment ion *m/z* 303.0473 (Figure S15, Supplementary Information section), whose presence has already been identified in the Connaraceae *R. cuspidata* Benth. ex Baker[6] and in crude extracts in *Connarus nodosus* Baker, *C. regnellii* and *C. suberosus*.[7] The molecular ion $[M + H]^+$ *m/z* 449.1050 is compatible with quercetin-3-*O*-rhamnoside (compound **16**), checked in CRLnb, CSLnb and RGLea, which was identified by the genin fragment ion *m/z* 303.0481 (Figure S16, Supplementary

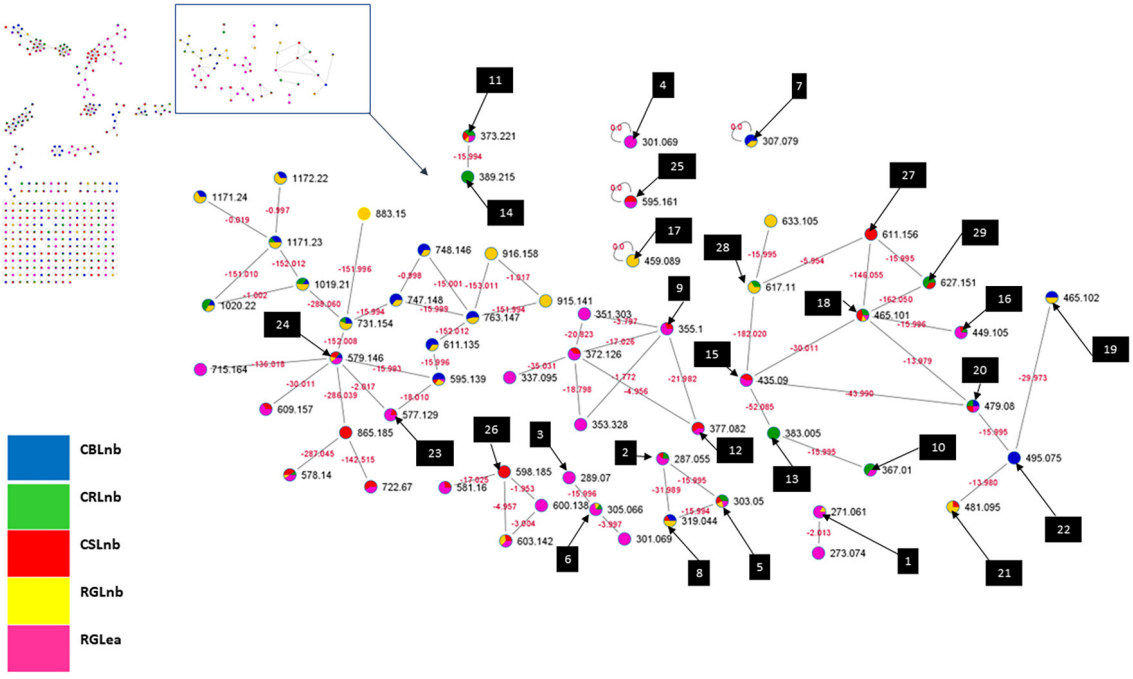

Figure 4. Clusters corresponding to chemical compounds identified. CBL = *C. blanchetii*, CRL = *C. regnellii*, CSL = *C. suberosus* and RGL = *R. glazioui*, nb = *n*-butanol and ea = ethyl acetate.

Information section). In quercetin-3-*O*-rhamnoside, two others fragment ions, *m/z* 153 and 287, measured in the mass spectrum are compatible with the predicted fragmentation of this metabolite. Through the molecular network, it is possible to verify that quercetin-3-*O*-rhamnoside is distanced from quercetin-3-*O*-galactoside (compound **18**), another quercetin derivative, by 16 Da, which is compatible with an oxygen atom. Quercetin-3-*O*-galactoside (Figure S18, Supplementary Information section) is present in CRLnb, CSLnb and RGLea/nb and was described in the Connaraceae *Rourea induta* Planch,[61] *R. cuspidata*[6] and *C. suberosus.*[7] Myricetin-3-*O*-rhamnoside (compound **19**), molecular ion $[M + H]^+$ *m/z* 465.1020, is one of the metabolites associated with CBLnb and RGLnb, being a glycosylated flavonoid derived from myricetin, with genin fragment ion *m/z* 319.0438 (Figure S19, Supplementary Information section). In our previous work,[7] we described myricetin-3-*O*-rhamnoside in crude extracts for *C. blanchetii* and *C. nodosus*. The other metabolites in this cluster were identified as quercetin-3-*O*-glucuronide, molecular ion $[M + H]^+$ *m/z* 479.0800, (compound **20**) for CBLnb, CRLnb, CSLnb and RGLea; myricetin-3-*O*-galactoside, molecular ion $[M + H]^+$ *m/z* 481.0950, (compound **21**) for CSLnb and RGLnb; myricetin-3-*O*-glucuronide, molecular ion $[M + H]^+$ *m/z* 495.0750, (compound **22**) for CBLnb; quercetin-3-*O*-rutinoside, molecular ion $[M + H]^+$ *m/z* 611.1560, (compound **27**) for CSLnb; quercetin 3-(2-galloylglucoside), molecular ion $[M + H]^+$ *m/z* 617.1100, (compound **28**) for CRLnb and RGLnb; and quercetin 3,4'-diglucoside, molecular ion $[M + H]^+$ *m/z* 627.1510, (compound **29**) for CRLnb and CSLnb.

Two other metabolites were identified as kaempferol-3-*O*-sulfate, $[M + H]^+$ *m/z* 367.0100, (compound **10**) and quercetin-3-*O*-sulphate, $[M + H]^+$ *m/z* 383.0050, (compound **13**), which form a cluster of two nodes whose mass difference is 16 Da. For these metabolites, the respective mass spectra produced the fragment ions *m/z* 287 (Figure S10, Supplementary Information section) and *m/z* 303 (Figure S13, Supplementary Information section), as the most intense, respectively.

In addition to the compounds already mentioned, several others could be identified among the species, the principal of which were: apigenin, $[M + H]^+$ *m/z* 271.0610, (compound **1**) for RGLea/nb; dihydroquercetin, $[M + H]^+$ *m/z* 305.0660, (compound **6**) for CRLnb and RGLea/nb; epigallocatechin, $[M + H]^+$ *m/z* 307.0790, (compound **7**) for CBLnb and RGLnb; chlorogenic acid, $[M + H]^+$ *m/z* 355.100, (compound **9**) for CSLnb and RGLea; protoanthocyanidin A1, $[M + H]^+$ *m/z* 577.1290, (compound **23**) for CSLnb and RGLea; and procyanidin B2, $[M + H]^+$ *m/z* 579.1460, (compound **24**) for CBLnb, CSLnb and RGLea/nb. In summary, the identity of 29 compounds among the different species of Connaraceae can be proposed from this work, with more information being available in Table 4 and in the Supplementary Information section.

Among the metabolites identified for these species of Connaraceae, several are implicated as promising molecules in the control of diabetes complications mediated by protein glycation and by the imbalance of redox metabolism. Derivatives of catechins,[76] quercetin, myricetin and apigenin[14,19,76] have already had their anti-AGEs effects demonstrated, and many of the compounds identified for these species of Connaraceae have already had their antioxidant effects reported by other authors.[61,77-80] Therefore, considering that hyperglycaemia results in an increase in the production of free radicals in diabetes, by a mechanism that involves the oxidation of glucose followed by the glycation of proteins,[81] and that the involvement of mitochondrial processes in the exacerbation of oxidative stress in response to hyperglycaemia is implicated with the complications of this disease,[82] the search for new therapeutic alternatives to reduce these complications is highlighted. In this context, among the species, *R. glazioui* has the broadest list of specialized metabolites that are potentially useful in the treatment of complications associated with diabetes, including the compounds apigenin, kaempferol, quercetin, myricetin, chlorogenic acid and others. Thus, the better performance against antiglycant activity observed for *R. glazioui* is probably associated with the synergistic effect of the phenolic compounds identified in this species, although the potential of other species (*C. blanchetii*, *C. regnellii*, and *C. suberosus*) cannot be overlooked. Plant therapies, with their multiple active metabolites, may in the future offer benefits in controlling diabetes complications and still have reduced toxicity.[83] Reviewing the literature, it is possible to infer that no antidiabetic drug to date has a reducing effect on protein glycation, so this is an alternative that still needs to be explored.

Conclusions

The results demonstrate that the *n*-butanolic fractions of the extracts showed the best antioxidant profile associated with these species. In the study of anti-AGE activities, the best result was seen for RGLea, with an IC_{50} of 36.5 μg mL^{-1} for oxidative pathway and 4.5 μg mL^{-1} for non-oxidative pathway. In summary, the identity of 29 compounds among C*onnarus blanchetii*, C*onnarus regnellii*, C*onnarus suberosus* and *Rourea glazioui* was achieved by the combined use of LC-MS analysis with bioinformatics

Table 4. Chemical composition of four Connaraceae species through LC-ESI-MS/MS in positive ion mode analysis identified by GNPS platform

Compound	t_R / min	Molecular formula	*m/z* exact	Error / ppm	Adduct	Fragmentation pathway *m/z* identified and fragment	Compound	Species fractions	Reference
1	67.2	$C_{15}H_{10}O_5$	271.0601	3.1	$[M + H]^+$	119 $[M – C_{11}H_3O]^+$, 145 $[M – C_5H_5]^+$, 153 $[M – C_8H_5O]^+$	apigenin	RGLnb, RGLea	62,63
2	66.8	$C_{15}H_{10}O_6$	287.0550	4.6	$[M + H]^+$	153 $[M – C_8H_5O_2]^+$, 269 $[M – OH]^+$	kaempferol	CRLnb, CSLnb, RGLea	9,64,65
3	65.6	$C_{15}H_{12}O_6$	289.0707	12.5	$[M + H]^+$	121 $[M – C_{12}H_7O]^+$, 153 $[M – C_8H_7O_2]^+$	dihydrokaempferol	RGLea	63
4	67.1	$C_{16}H_{12}O_6$	301.0707	7.1	$[M + H]^+$	153 $[M – C_9H_7O_2]^+$	chrysoeriol	RGLea	
5	65.0	$C_{15}H_{10}O_7$	303.0499	6.0	$[M + H]^+$	137 $[M – C_8H_5O_4]^+$, 153 $[M – C_8H_5O_3]^+$, 257 $[M – CHO_2]^+$	quercetin	CRLnb, CSLnb, RGLnb, RGLea	65,66
6	64.7	$C_{15}H_{12}O_7$	305.0656	5.2	$[M + H]^+$	123 $[M – C_8H_5O_5]^+$, 153 $[M – C_8H_7O_3]^+$	dihydroquercetin	CRLnb, RGLnb, RGLea	63,67
7	25.2	$C_{15}H_{14}O_7$	307.0812	13.0	$[M + H]^+$	139 $[M – C_{12}H_7O]^+$, 151 $[M – C_9H_7O]^+$, 163 $[M – C_6H_7O_4]^+$	epigallocatechin	CBLnb, RGLnb	68
8	65.2	$C_{15}H_{10}O_8$	319.0448	3.7	$[M + H]^+$	153 $[M – C_8H_5O_4]^+$, 165 $[M – C_{11}H_5O]^+$, 217 $[M – C_3HO_4]^+$, 273 $[M – CHO_2]^+$	myricetin	CBLnb, CSLnb, RGLnb	65
9	31.7	$C_{16}H_{18}O_9$	355.1024	7.5	$[M + H]^+$	135 $[M – C_8H_{11}O_7]^+$, 145 $[M – C_{11}H_{13}O_4]^+$, 163 $[M – C_7H_{11}O_6]^+$	chlorogenic acid	CSLnb, RGLea	69
10	65.3	$C_{15}H_{10}O_9S$	367.0118	5.9	$[M + H]^+$	153 $[M – C_8H_5O_5S]^+$, 287 $[M – SO_3]^+$	kaempferol-3-*O*-sulfate	CRLnb, RGLea	
11	66.0	$C_{19}H_{32}O_7$	373.2221	5.6	$[M + H]^+$	151 $[M – C_{15}H_{25}O]^+$, 175 $[M – C_{10}H_{13}O_4]^+$, 193 $[M – C_6H_{11}O_6]^+$, 211 $[M – C_6H_9O_5]^+$	3,5,5-trimethyl-4-[3-(beta-D-glucopyranosyloxy) butyl]-2-cyclohexene-1-one	CRLnb, CSLnb, RGLnb, RGLea	
12	32.4	$C_{16}H_{18}NaO_9$	377. 0853	9.0	$[M + Na]^+$	163 $[M – C_6H_{11}O_6Na]^+$	(3*R*,5*S*)-4-[(*E*)-3-(3,4-di-hydroxyphenyl)prop-2- enoyl]oxy-1,3,5-trihydro-xycyclohexane-1-carboxylic acid	CSLnb, RGLea	
13	65.1	$C_{15}H_{10}O_{10}S$	383.0067	6.2	$[M + H]^+$	257 $[M – CHO_5S]^+$, 303 $[M – SO_3]^+$	quercetin-3-*O*-sulfate	CRLnb	70
14	64.4	$C_{19}H_{32}O_8$	389.2170	8.1	$[M + H]^+$	209 $[M – C_6H_{11}O_6]^+$	icariside B5	CRLnb	
15	66.0	$C_{20}H_{18}O_{11}$	435.0922	8.0	$[M + H]^+$	153 $[M – C_{13}H_{13}O_{11}]^+$, 285 $[M – C_9H_9O_2]^+$, 303$[M – C_5H_7O_4]^+$	quercetin-3-*O*-pentoside (guaijaverin)	CSLnb, RGLea	71
16	66.2	$C_{21}H_{20}O_{11}$	449.1078	4.0	$[M + H]^+$	129 $[M – C_{16}H_{15}O_7]^+$, 153 $[M – C_{14}H_{15}O_7]^+$, 287 $[M – C_6H_9O_5]^+$, 303 $[M – C_6H_9O_4]^+$	quercetin-3-*O*-rhamnoside (quercitrin)	CRLnb, CSLnb, RGLea	70,72
17	65.6	$C_{22}H_{18}O_{11}$	459.0922	12.0	$[M + H]^+$	123 $[M – C_{15}H_{11}O_5]^+$, 153 $[M – C_{15}H_{13}O_7]^+$	epigallocatechin gallate	RGLnb	73
18	65.8	$C_{21}H_{20}O_{12}$	465.1028	4.7	$[M + H]^+$	145 $[M – C_{16}H_{15}O_7]^+$, 153 $[M – C_{14}H_{15}O_8]^+$, 247 $[M – C_{12}H_9O_4]^+$, 275 $[M – C_{11}H_9O_5]^+$, 285 $[M – C_{10}H_{11}O_3]^+$, 303 $[M – C_6H_9O_5]^+$	quercetin-3-*O*-galactoside (hyperin)	CRLnb, CSLnb, RGLnb, RGLea	6,72

Table 4. Chemical composition of four Connaraceae species through LC-ESI-MS/MS in positive ion mode analysis identified by GNPS platform (cont.)

Compound	t_R / min	Molecular formula	*m/z* exact	Error / ppm	Adduct	Fragmentation pathway *m/z* identified and fragment	Compound	Species fractions	Reference
19	65.6	$C_{21}H_{20}O_{12}$	465.1028	1.8	$[M + H]^+$	129 $[M - C_{15}H_{11}O_9]^+$, 153 $[M - C_{14}H_{15}O_8]^+$, 273 $[M - C_7H_{11}O_6]^+$, 301 $[M - C_6H_{11}O_5]^+$, 319 $[M - C_6H_9O_4]^+$	myricetin-3-*O*-rhamnoside (myricitrin)	CBLnb, RGLnb	70
20	65.7	$C_{21}H_{18}O_{13}$	479.0820	4.0	$[M + H]^+$	153 $[M - C_{14}H_{13}O_9]^+$, 159 $[M - C_{15}H_{11}O_8]^+$, 247 $[M - C_{12}H_7O_5]^+$, 273 $[M - C_7H_9O_7]^+$, 303 $[M - C_6H_7O_6]^+$	quercetin-3-*O*-glucuronide (quercituron)	CBLnb, CRLnb, CSLnb, RGLea	70,71,74
21	65.5	$C_{21}H_{20}O_{13}$	481.0977	9.0	$[M + H]^+$	303 $[M - C_6H_9O_6]^+$, 319 $[M - C_6H_9O_5]^+$	myricetin-3-*O*-galactoside	CSLnb, RGLnb	74
22	65.5	$C_{21}H_{18}O_{14}$	495.0769	1.9	$[M + H]^+$	153 $[M - C_{14}H_{13}O_{10}]^+$, 159 $[M - C_{15}H_{11}O_9]^+$, 301 $[M - C_6H_9O_7]^+$, 319 $[M - C_6H_7O_6]^+$.	myricetin-3-*O*-glucuronide	CBLnb	70
23	65.5	$C_{30}H_{24}O_{12}$	577.1341	4.5	$[M + H]^+$	139 $[M - C_{27}H_{17}O_6]^+$, 425 $[M - C_{12}H_7]^+$	proanthocyanidin A1	CSLnb, RGLea	6
24	40.1	$C_{30}H_{26}O_{12}$	578.1424	8.1	$[M + H]^+$	139 $[M - C_{27}H_{19}O_6]^+$, 151 $[M - C_{26}H_{19}O_6]^+$, 257 $[M - C_{16}H_{17}O_7]^+$, 275 $[M - C_{20}H_{15}O_3]^+$, 287 $[M - C_{15}H_{15}O_6]^+$	procyanidin B2	CBLnb, CRLnb, CSLnb, RGLnb, RGLea	75
25	64.6	$C_{27}H_{30}O_{15}$	595.1657	6.8	$[M + H]^+$	385 $[M - C_{15}H_{13}O_{11}]^+$, 559 $[M - H_3O_2]^+$	5,7-dihydroxy-3-(4-hydroxyphenyl)-6,8-bis[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] chromen-4-one	CSLnb, RGLea	
26	59.6	$C_{30}H_{32}NO_{12}$	598.1918	14.9	$[2M + NH_4]+$	123 $[M - C_7H_{25}NO_{10}]^+$, 139 $[M - C_{27}H_{25}NO_6]^+$, 291 $[M - C_{15}H_{17}NO_6]^+$	epicatechin	CSLnb	
27	65.9	$C_{27}H_{30}O_{16}$	611.1607	6.9	$[M + H]^+$	153 $[M - C_{20}H_{25}O_{12}]^+$, 285 $[M - C_{16}H_{21}O_7]^+$, 303 $[M - C_{12}H_{19}O_9]^+$	quercetin-3-*O*-rutinoside (tutin)	CSLnb	70,71
28	65.6	$C_{28}H_{24}O_{16}$	617.1137	18.6	$[M + H]^+$	153 $[M - C_{21}H_{19}O_{12}]^+$, 303 $[M - C_{13}H_{13}O_9]^+$	quercetin 3-(2-galloylglucoside)	CRLnb, RGLnb	70
29	65.2	$C_{27}H_{30}O_{17}$	627.1556	5.3	$[M + H]^+$	303 $[M - C_{12}H_{19}O_{10}]^+$	quercetin diglucoside	CRLnb, CSLnb	70

CBL: *C. blanchetii*; CRL: *C. regnellii*; CSL: *C. suberosus*; RGL: *R. glazioui*; nb: *n*-butanol; ea: ethyl acetate.

tools. *Rourea glazioui* has the broadest list of specialized metabolites that are potentially useful in the treatment of complications associated with diabetes, including the compounds apigenin, kaempferol, quercetin, myricetin and chlorogenic acid although the potential of other species cannot be overlooked. In summary, this work demonstrates a scientific search confirming that the popular use of these plants species is still little explored, and highlights that these plant species can bring an excellent response to one of the problems that most affects humanity in modern daily life, diabetes.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

This study was financially supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), under Finance Code 001, which also provided a doctoral fellowship to the second author (process number 88882.329252/2018-01), and Fundação de Amparo à

Pesquisa do Estado de São Paulo (FAPESP), under process number 2019/03173-0, both Brazilian institutions.

Author Contributions

Luis Fernando N. A. Paim and Paulo R. dos Santos conducted the chemical and biological analyses and wrote the manuscript. Cássio A. P. Toledo and Joicelene R. L. da Paz collected and identified the plants and assisted in the modification and adaptation of the text. Luana Minello assisted in the modification and adaptation of the text. Leonard D. R. Acho performed the anti-AGEs assays. Sidnei Moura, Mirian Salvador and Emerson Lima made the final revision of the manuscript. All authors approved the final submitted version of the manuscript.

References

1. Lemmens, R. H. M. J.; Breteler, F. J.; Jongkind, C. C. H. In *Flowering Plants Dicotyledons*; Kubitzki, K., ed.; Springer: Berlin, 2004, p. 74. [Crossref]
2. Paim, L. F.; Patrocínio Toledo, C. A.; Lima da Paz, J. R.; Picolotto, A.; Ballardin, G.; Souza, V. C.; Salvador, M.; Moura, S.; *J. Ethnopharmacol.* **2020**, *261*, 112980. [Crossref]
3. Adisa, R. A.; Choudhary, M. I.; Adewoye, E. O.; Olorunsogo, O. O.; *Afr. J. Tradit., Complementary Altern. Med.* **2010**, *7*, 185. [Crossref]
4. Dada, O. K.; Akindele, A. J.; Morakinyo, O. A.; Sofidiya, M. O.; Ota, D.; *Chin. J. Nat. Med.* **2013**, *11*, 628. [Crossref]
5. Kulkarni, P.; Patel, V.; Shukla, S. T.; Patel, A.; Kulkarni, V.; *Orient. Pharm. Exp. Med.* **2014**, *14*, 69. [Crossref]
6. Laikowski, M. M.; dos Santos, P. R.; Souza, D. M.; Minetto, L.; Girondi, N.; Pires, C.; Alano, G.; Roesch-Ely, M.; Tasso, L.; Moura, S.; *Asian Pac. J. Trop. Biomed.* **2017**, *7*, 712. [Crossref]
7. Paim, L. F. N. A.; dos Santos, P. R.; Toledo, C. A. P.; Minello, L.; da Paz, J. R. L.; Souza, V. C.; Salvador, M.; Moura, S.; *Phytochem. Anal.* **2021**, *33*, 286. [Crossref]
8. Akindele, A. J.; Iyamu, E. A.; Dutt, P.; Satti, N. K.; Adeyemi, O. O.; *Afr. J. Tradit., Complementary Altern. Med.* **2014**, *4*, 177. [Crossref]
9. Aryal, B.; Niraula, P.; Khadayat, K.; Adhikari, B.; Khatri Chhetri, D.; Sapkota, B. K.; Bhattarai, B. R.; Aryal, N.; Parajuli, N.; *Afr. J. Tradit., Complement. Altern. Med.* **2021**, *2021*, 5510099. [Crossref]
10. Adisa, R. A.; Oke, J. M.; Olomu, S. A.; Olorunsogo, O. O.; *J. Cameroon Acad. Sci.* **2004**, *4*, 351. [Link] accessed in May 2023
11. Negri, G.; *Rev. Bras. Cienc. Farm.* **2005**, *41*, 121. [Crossref]
12. Asmat, U.; Abad, K.; Ismail, K.; *Saudi Pharm. J.* **2016**, *24*, 547. [Crossref]
13. Scott, J. A.; King, G. L.; *Ann. N. Y. Acad. Sci.* **2004**, *1031*, 204. [Crossref]
14. Wu, C.-H.; Yen, G.-C.; *J. Agric. Food Chem.* **2005**, *53*, 3167. [Crossref]
15. Esteghamati, A.; Eskandari, D.; Mirmiranpour, H.; Noshad, S.; Mousavizadeh, M.; Hedayati, M.; Nakhjavani, M.; *Clin. Nutr.* **2013**, *32*, 179. [Crossref]
16. Barbosa, J. H. P.; Oliveira, S. L.; Seara, L. T.; *Arq. Bras. Endocrinol. Metabol.* **2008**, *52*, 940. [Crossref]
17. Kim, H. Y.; Lee, J. M.; Yokozawa, T.; Sakata, K.; Lee, S.; *Food Chem.* **2011**, *126*, 892. [Crossref]
18. Byun, K.; Yoo, Y.; Son, M.; Lee, J.; Jeong, G.-B.; Park, Y. M.; Salekdeh, G. H.; Lee, B.; *Pharmacol. Ther.* **2017**, *177*, 44. [Crossref]
19. Bains, Y.; Gugliucci, A.; *Fitoterapia* **2017**, *117*, 6. [Crossref]
20. Ahmadu, A. A.; Hassan, H. S.; Abubakar, M. U.; Akpulu, I. N.; *Afr. J. Tradit., Complementary Altern. Med.* **2007**, *4*, 257. [Crossref]
21. Kalegari, M.; Cerutti, M. L.; Macedo-Júnior, S. J.; Bobinski, F.; Miguel, M. D.; Eparvier, V.; Santos, A. R. S.; Stien, D.; Miguel, O. G.; *J. Ethnopharmacol.* **2014**, *153*, 801. [Crossref]
22. Pires, F. B.; Dolwitsch, C. B.; Dal Prá, V.; Faccin, H.; Monego, D. L.; de Carvalho, L. M.; Viana, C.; Lameira, O.; Lima, F. O.; Bressan, L.; da Rosa, M. B.; *Rev. Bras. Farmacogn.* **2017**, *27*, 426. [Crossref]
23. SisGen, *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado*, https://sisgen.gov.br/paginas/login.aspx, accessed in April 2023.
24. Oliveira, V. B.; Zuchetto, M.; Oliveira, C. F.; Paula, C. S.; Duarte, A. F. S.; Miguel, M. D.; Miguel, O. G.; *Rev. Bras. Plantas Med.* **2016**, *18*, 230. [Crossref]
25. Ayouni, K.; Berboucha-Rahmani, M.; Kim, H. K.; Atmani, D.; Verpoorte, R.; Choi, Y. H.; *Ind. Crops Prod.* **2016**, *88*, 65. [Crossref]
26. Gomes, S. V. F.; Portugal, L. A.; dos Anjos, J. P.; de Jesus, O. N.; de Oliveira, E. J.; David, J. P.; David, J. M.; *Microchem. J.* **2017**, *132*, 28. [Crossref]
27. Janovik, V.; Boligon, A.; Feltrin, A.; Pereira, D.; Frohlich, J.; Linde Athayde, M.; *Rev. Cent. Cienc. Saude* **2009**, *35*, 25. [Crossref]
28. Haida, S.; Kribii, A.; Kribii, A.; *S. Afr. J. Bot.* **2020**, *131*, 151. [Crossref]
29. Leitão, S. G.; Castro, O.; Fonseca, E. N.; Julião, L. S.; Tavares, E. S.; Leo, R. R. T.; Vieira, R. C.; Oliveira, D. R.; Leitão, G. G.; Martino, V.; Sulsen, V.; Barbosa, Y. A. G.; Pinheiro, D. P. G.; da Silva, P. E. A.; Teixeira, D. F.; N. Junior, I.; Lourenço, M. C. S.; *Rev. Bras. Farmacogn.* **2006**, *16*, 6. [Crossref]
30. Sousa, C. M. M.; Silva, H. R.; Vieira-Jr., G. M.; Ayres, M. C. C.; da Costa, C. L. S.; Araújo, D. S.; Cavalcante, L. C. D.; Barros, E. D. S.; Araújo, P. B. M.; Brandão, M. S.; Chaves, M. H.; *Quim. Nova* **2007**, *30*, 351. [Crossref]
31. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Hawkins Byrne, D.; *J. Food Compos. Anal.* **2006**, *19*, 669. [Crossref]

32. Pulido, R.; Bravo, L.; Saura-Calixto, F.; *J. Agric. Food Chem.* **2000**, *48*, 3396. [Crossref]
33. Kiho, T.; Usui, S.; Hirano, K.; Aizawa, K.; Inakuma, T.; *Biosci., Biotechnol., Biochem.* **2004**, *68*, 200. [Crossref]
34. Oliveira, E. S. C.; Pontes, F. L. D.; Acho, L. D. R.; do Rosário, A. S.; da Silva, B. J. P.; Bezerra, J. A.; Campos, F. R.; Lima, E. S.; Machado, M. B.; *J. Pharm. Biomed. Anal.* **2021**, *201*, 114109. [Crossref]
35. Wang, M.; Carver, J. J.; Vanessa V. Phelan; Sanchez, L. M.; Garg, N.; Peng, Y.; Nguyen, D. D.; Watrous, J.; Kapono, C. A.; Luzzatto-Knaan, T.; Porto, C.; Bouslimani, A.; Melnik, A. V.; Meehan, M. J.; Liu, W.-T.; Crüsemann, M.; Boudreau, P. D.; Esquenazi, E.; Sandoval-Calderón, M.; Kersten, R. D.; Pace, L. A.; Quinn, R. A.; Duncan, K. R.; Hsu, C.-C.; Floros, D. J.; Gavilan, R. G.; Kleigrewe, K.; Northen, T.; Dutton, R. J.; Parrot, D.; Carlson, E. E.; Aigle, B.; Michelsen, C. F.; Jelsbak, L.; Sohlenkamp, C.; Pevzner, P.; Edlund, A.; McLean, J.; Piel, J.; Murphy, B. T.; Gerwick, L.; Liaw, C.-C.; Yang, Y.-L.; Humpf, H.-U.; Maansson, M.; Keyzers, R. A.; Sims, A. C.; Johnson, A. R.; Sidebottom, A. M.; Sedio, B. E.; Klitgaard, A.; Larson, C. B.; Boya P, C. A.; Torres-Mendoza, D.; Gonzalez, D. J.; Silva, D. B.; Marques, L. M.; Demarque, D. P.; Pociute, E.; O'Neill, E. C.; Briand, E.; Helfrich, E. J. N.; Granatosky, E. A.; Glukhov, E.; Ryffel, F.; Houson, H.; Mohimani, H.; Kharbush, J. J.; Zeng, Y.; Vorholt, J. A.; Kurita, K. L.; Charusanti, P.; McPhail, K. L.; Nielsen, K. F.; Vuong, L.; Elfeki, M.; Traxler, M. F.; Engene, N.; Koyama, N.; Vining, O. B.; Baric, R.; Silva, R. R.; Mascuch, S. J.; Tomasi, S.; Jenkins, S.; Macherla, V.; Hoffman, T.; Agarwal, V.; Williams, P. G.; Dai, J.; Neupane, R.; Gurr, J.; Rodríguez, A. M. C.; Lamsa, A.; Zhang, C.; Dorrestein, K.; Duggan, B. M.; Almaliti, J.; Allard, P.-M.; Phapale, P.; Nothias, L.-F.; Alexandrov, T.; Litaudon, M.; Wolfender, J.-L.; Kyle, J. E.; Metz, T. O.; Peryea, T.; Nguyen, D.-T.; VanLeer, D.; Shinn, P.; Jadhav, A.; Müller, R.; Waters, K. M.; Shi, W.; Liu, X.; Zhang, L.; Knight, R.; Jensen, P. R.; Palsson, B. Ø.; Pogliano, K.; Linington, R. G.; Gutiérrez, M.; Lopes, N. P.; Gerwick, W. H.; Moore, B. S.; Dorrestein, P. C.; Bandeira, N.; *Nat. Biotechnol.* **2016**, *34*, 828. [Crossref]
36. Santos, A. L.; Soares, M. G.; de Medeiros, L. S.; Ferreira, M. J. P.; Sartorelli, P.; *Phytochem. Anal.* **2021**, *32*, 891. [Crossref]
37. Cytoscape, https://cytoscape.org/, accessed in April 2023.
38. CFM-ID: Spectra Prediction, https://cfmid.wishartlab.com/predict, accessed in April 2023.
39. Allen, F.; Pon, A.; Wilson, M.; Greiner, R.; Wishart, D.; *Nucleic Acids Res.* **2014**, *42*, 94. [Crossref]
40. Djoumbou-Feunang, Y.; Pon, A.; Karu, N.; Zheng, J.; Li, C.; Arndt, D.; Gautam, M.; Allen, F.; Wishart, D. S.; *Metabolites* **2019**, *9*, 72. [Crossref]
41. PubChem, https://pubchem.ncbi.nlm.nih.gov/, accessed in April 2023.
42. *ChemCalc: Molecular Formula Information*, https://www.chemcalc.org/, accessed in April 2023.
43. Catarino, L.; Havik, P. J.; Romeiras, M. M.; *J. Ethnopharmacol.* **2016**, *183*, 71. [Crossref]
44. Henkin, J. M.; Sydara, K.; Xayvue, M.; Souliya, O.; Kinghorn, A. D.; Burdette, J. E.; Chen, W.-L.; Elkington, B. G.; Soejarto, D. D.; *J. Med. Plants Res.* **2017**, *11*, 621. [Crossref]
45. Pedrollo, C. T.; Kinupp, V. F.; Shepard, G.; Heinrich, M.; *J. Ethnopharmacol.* **2016**, *186*, 111. [Crossref]
46. Moura, V. M.; Freitas de Sousa, L. A.; Cristina Dos-Santos, M.; Almeida Raposo, J. D.; Evangelista Lima, A.; de Oliveira, R. B.; da Silva, M. N.; Veras Mourão, R. H.; *J. Ethnopharmacol.* **2015**, *161*, 224. [Crossref]
47. Sabran, S. F.; Mohamed, M.; Bakar, M. F. A.; *J. Evidence-Based Complementary Altern. Med.* **2016**, *2016*, ID 2850845. [Crossref]
48. Tchicaillat-Landou, M.; Petit, J.; Gaiani, C.; Miabangana, E. S.; Kimbonguila, A.; Nzikou, J.-M.; Scher, J.; Matos, L.; *J. Herb. Med.* **2018**, *13*, 76. [Crossref]
49. Novy, J. W.; *J. Ethnopharmacol.* **1997**, *55*, 119. [Crossref]
50. Mesia, G. K.; Tona, G. L.; Nanga, T. H.; Cimanga, R. K.; Apers, S.; Cos, P.; Maes, L.; Pieters, L.; Vlietinck, A. J.; *J. Ethnopharmacol.* **2008**, *115*, 409. [Crossref]
51. Veber, J.; Petrini, L. A.; Andrade, L. B.; Siviero, J.; Veber, J.; Petrini, L. A.; Andrade, L. B.; Siviero, J.; *Rev. Bras. Plantas Med.* **2015**, *17*, 267. [Crossref]
52. Cowan, M. M.; *Clin. Microbiol. Rev.* **1999**, *12*, 564. [Crossref]
53. Ahmed, D.; Fatima, M.; Saeed, S.; *Asian Pac. J. Trop. Med.* **2014**, *7*, 249. [Crossref]
54. Belhaoues, S.; Amri, S.; Bensouilah, M.; *S. Afr. J. Bot.* **2020**, *131*, 200. [Crossref]
55. Besbas, S.; Mouffouk, S.; Haba, H.; Marcourt, L.; Wolfender, J.-L.; Benkhaled, M.; *Phytochem. Lett.* **2020**, *37*, 63. [Crossref]
56. Moon, J.-K.; Shibamoto, T.; *J. Agric. Food Chem.* **2009**, *57*, 1655. [Crossref]
57. Antolovich, M.; Prenzler, P. D.; Patsalides, E.; McDonald, S.; Robards, K.; *Analyst* **2002**, *127*, 183. [Crossref]
58. Sobottka, A. M.; Tessaro, E.; da Silva, S. M.; Pedron, M.; Seffrin, L. T.; *Rev. Árvore* **2021**, *45*, e4507. [Crossref]
59. Ramos, A. S.; Mar, J. M.; da Silva, L. S.; Acho, L. D. R.; Silva, B. J. P.; Lima, E. S.; Campelo, P. H.; Sanches, E. A.; Bezerra, J. A.; Chaves, F. C. M.; Campos, F. R.; Machado, M. B.; *Food Res. Int.* **2019**, *123*, 674. [Crossref]
60. Patiny, L.; Borel, A.; *J. Chem. Inf. Model.* **2013**, *53*, 1223. [Crossref]
61. Kalegari, M.; Gemin, C. A. B.; Araújo-Silva, G.; de Brito, N. J. N.; López, J. A.; Tozetto, S. O.; das Graças Almeida, M.; Miguel, M. D.; Stien, D.; Miguel, O. G.; *Nutrition* **2014**, *30*, 713. [Crossref]
62. Dou, X.; Zhou, Z.; Ren, R.; Xu, M.; *Biomed. Pharmacother.* **2020**, *128*, 110298. [Crossref]

63. Razgonova, M.; Zakharenko, A.; Pikula, K.; Manakov, Y.; Ercisli, S.; Derbush, I.; Kislin, E.; Seryodkin, I.; Sabitov, A.; Kalenik, T.; Golokhvast, K.; *Molecules* **2021**, *26*, 3650. [Crossref]
64. March, R. E.; Miao, X.-S.; *Int. J. Mass Spectrom.* **2004**, *231*, 157. [Crossref]
65. Wolfender, J.-L.; Waridel, P.; Ndjoko, K.; Hobby, K. R.; Major, H. J.; Hostettmann, K.; *Analusis* **2000**, *28*, 895. [Crossref]
66. Singh, P.; Bajpai, V.; Gupta, A.; Gaikwad, A. N.; Maurya, R.; Kumar, B.; *Ind. Crops Prod.* **2019**, *127*, 26. [Crossref]
67. Yang, P.; Xu, F.; Li, H.-F.; Wang, Y.; Li, F.-C.; Shang, M.-Y.; Liu, G.-X.; Wang, X.; Cai, S.-Q.; *Molecules* **2016**, *21*, 1209. [Crossref]
68. Umehara, M.; Yanae, K.; Maruki-Uchida, H.; Sai, M.; *Food Res. Int.* **2017**, *102*, 77. [Crossref]
69. Palierse, E.; Przybylski, C.; Brouri, D.; Jolivalt, C.; Coradin, T.; *Int. J. Mol. Sci.* **2020**, *21*, 4948. [Crossref]
70. Santos, M. C.; Toson, N. S. B.; Pimentel, M. C. B.; Bordignon, S. A. L.; Mendez, A. S. L.; Henriques, A. T.; *J. Ethnopharmacol.* **2020**, *255*, 112781. [Crossref]
71. Mari, A.; Lyon, D.; Fragner, L.; Montoro, P.; Piacente, S.; Wienkoop, S.; Egelhofer, V.; Weckwerth, W.; *Metabolomics* **2013**, *9*, 599. [Crossref]
72. Jang, G.-H.; Kim, H. W.; Lee, M. K.; Jeong, S. Y.; Bak, A. R.; Lee, D. J.; Kim, J. B.; *Saudi J. Biol. Sci.* **2018**, *25*, 1622. [Crossref]
73. Susanti, E.; Ciptati; Ratnawati, R.; Aulanni'am; Rudijanto, A.; *Asian Pac. J. Trop. Biomed.* **2015**, *5*, 1046. [Crossref]
74. Rezende, F. M.; Ferreira, M. J. P.; Clausen, M. H.; Rossi, M.; Furlan, C. M.; *Molecules* **2019**, *24*, 718. [Crossref]
75. Liu, P.; Yang, B.; Kallio, H.; *Food Chem.* **2010**, *121*, 1188. [Crossref]
76. Zhu, Z.; Bassey, A. P.; Khan, I. A.; Huang, M.; Zhang, X.; *LTW - Food Sci. Technol.* **2021**, *147*, 111550. [Crossref]
77. Ahmed, S.; Al-Rehaily, A. J.; Alam, P.; Alqahtani, A. S.; Hidayatullah, S.; Rehman, Md. T.; Mothana, R. A.; Abbas, S. S.; Khan, M. U.; Khalid, J. M.; Siddiqui, N. A.; *Saudi Pharm. J.* **2019**, *27*, 655. [Crossref]
78. Domitrović, R.; Rashed, K.; Cvijanović, O.; Vladimir-Knežević, S.; Škoda, M.; Višnić, A.; *Chem.-Biol. Interact.* **2015**, *230*, 21. [Crossref]
79. Grzesik, M.; Naparło, K.; Bartosz, G.; Sadowska-Bartosz, I.; *Food Chem.* **2018**, *241*, 480. [Crossref]
80. Tian, C.; Liu, X.; Chang, Y.; Wang, R.; Lv, T.; Cui, C.; Liu, M.; *S. Afr. J. Bot.* **2021**, *137*, 257. [Crossref]
81. Maritim, A. C.; Sanders, R. A.; Watkins III, J. B.; *J. Biochem. Mol. Toxicol.* **2003**, *17*, 24. [Crossref]
82. Ceriello, A.; Ihnat, M. A.; Thorpe, J. E.; *J. Clin. Endocrinol. Metab.* **2009**, *94*, 410. [Crossref]
83. Ha, D. T.; Ngoc, T. M.; Lee, I.; Lee, Y. M.; Kim, J. S.; Jung, H.; Lee, S.; Na, M.; Bae, K.; *J. Nat. Prod.* **2009**, *72*, 1465. [Crossref]

Submitted: November 3, 2022
Published online: May 9, 2023