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Article

New Isoflavones from the Leaves of *Vatairea guianensis* Aublé

Ronilson Freitas de Souza,[a] Victor H. S. Marinho,[a] Geilson A. da Silva,[a] Livio M. Costa-Jr.,[b] Joyce Kelly R. da Silva,[c] Gilmara N. T. Bastos,[d] Alberto C. Arruda,[a] Milton N. da Silva[a] and Mara Silvia P. Arruda*[,a]

[a]Programa de Pós-Graduação em Química, Instituto de Ciências Exatas e Naturais, Universidade Federal do Pará, Campus Universitário do Guamá, 66075-970 Belém-PA, Brazil

[b]Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão, MA-230, km 04, s/n, Boa Vista, 65500-000 Chapadinha-MA, Brazil

[c]Programa de Pós-Graduação em Biotecnologia and [d]Programa de Pós-Graduação em Neurociências e Biologia Celular, Instituto de Ciências Biológicas, Universidade Federal do Pará, Campus Universitário do Guamá, 66075-970 Belém-PA, Brazil

Das folhas de *Vatairea guianensis* Aublé foram isoladas quatro isoflavonas identificadas como, 5,3',-diidroxi-4'-metoxi-2",2"-dimetilpirano-(5",6":8,7)-isoflavona (**1**), 5,7-diidroxi-3',4'-metilenodioxi-8-prenil-isoflavona (**2**) e 5,3'-diidroxi-4'-metoxi-7-*O*-β-glicopiranosídeo-8-prenil-isoflavona (**3**) e derrona (**4**), juntamente com cinco triterpenos identificados em mistura de lupeol, α-amirina, β-amirina, germanicol e ácido betulínico. As substâncias **1**-**3** são novos produtos naturais, porém **1** e **2** já foram citados como produtos de síntese. No entanto, todas essas substâncias são relatadas pela primeira vez para essa espécie. Suas estruturas químicas foram elucidadas com base nos seus dados de ressonância magnética nuclear (RMN) 1D e 2D e por espectrometria de massas de alta resolução. O extrato etanólico das folhas e os compostos **1-3** foram avaliados quanto ao seu potencial sequestrador do radical DPPH• (2,2-difenil-1-picril-hidrazila) e os resultados mostram que o extrato apresentou alta atividade (CI_{50} = 6,2 ± 0,4 μg mL^{-1}), enquanto as substâncias testadas apresentaram baixo poder antioxidante (CI_{50} ≥ 29,5 ± 2,5 μg mL^{-1}) quando comparadas com TROLOX (CI_{50} = 4,5 ± 0,4 μg mL^{-1}).

Four isoflavones were isolated from *Vatairea guianensis* Aublé leaves and identified as 5,3'-dihydroxy-4'-methoxy-2",2"-dimethylpyrano-(5",6":8,7)-isoflavone (**1**), 5,7-dihydroxy-3',4'-methylenedioxy-8-prenyl-isoflavone (**2**), 5,3'-dihydroxy-4'-methoxy-7-*O*-β-glucopyranoside-8-prenyl-isoflavone (**3**) and derrone (**4**) together with five triterpenes identified in mixture, lupeol, α-amyrin, β-amyrin, germanicol and betulinic acid. Substances **1**-**3** are novel natural products, although **1** and **2** have been cited as synthetic products. However, all these compounds are first reported from this species. Their chemical structures were elucidated based on their 1D and 2D nuclear magnetic resonance (NMR) data and high resolution mass spectrometry. The ethanol extract from the leaves and **1**-**3** were evaluated for their potential in scavenging DPPH• (2,2-diphenyl-1-picrylhydrazylradical) and the results showed that the extract presented high activity (IC_{50} = 6.2 ± 0.4 μg mL^{-1}), while the isolated compounds showed low antioxidant power (IC_{50} ≥ 29.5 ± 2.5 μg mL^{-1}) when compared to Trolox (IC_{50} = 4.5 ± 0.4 μg mL^{-1}).

Keywords: *Vatairea guianensis*, Fabaceae, isoflavones, antioxidant activity, DPPH•

Introduction

The genus *Vatairea* (Fabaceae) includes seven species distributed in Brazil, the Guianas and the Atlantic coastal regions of Central America and Mexico.[1] The species *Vatairea guianensis* Aublé is a plant native to the Amazon, known as "fava bolacha" or "fava de impingem".[2] Its fruits, bark from the stems, roots and leaves are commonly used for treating surface micoses.[3,4] Few scientific references citing biological activities for this species have been found in the literature. Fruits are the only part of the plant that has been studied in terms of biological potential, presenting *in vitro*

*e-mail: mspa@ufpa.br

action against *Leishmania amazonensis*,[5] antimicrobial[6] and topical healing activity.[7] Among the components reported for the plant are antraquinones chrysophanol, physcion and emodin, antrones 9-anthronechrysophanol acid, 9-anthronephyscion and 10-anthronephyscion, triterpenes oleanolic acid and dihydromacaerinic acid lactone.[4,8] These reports of phytochemical studies are restricted to fruits, heartwood and bark of the stems. The leaves of *V. guianensis* were chosen for this study in an effort to identify their chemical composition and assess their potential for scavenging radical DPPH•. Four isoflavones were isolated (**1**-**4**) (Figure 1) together with a mixture of known triterpenes lupeol, α-amyrin, β-amyrin, germanicol and betulinic acid. The ethanolic extract of the leaves and isoflavones **1-3** were evaluated for their potential to scavenge DPPH•.

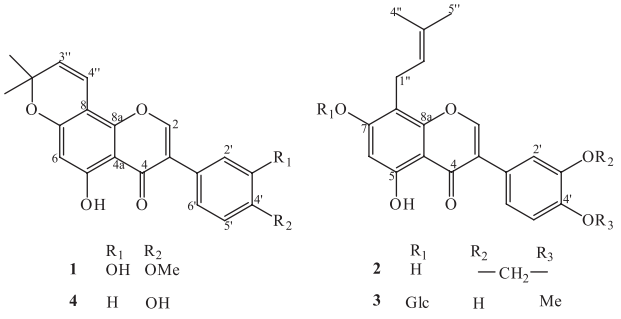

Figure 1. Structures of the isoflavones isolated from *V. guianensis*.

Experimental

General

UV spectra were obtained from a liquid chromatograph (LC) equipped with diode array detection (DAD) Prominence SPDM-20A (Shimadzu, Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra including 1D and 2D experiments were recorded on a Varian Mercury-300 spectrometer, operating at 300 MHz at ^{1}H and 75 MHz at ^{13}C, using $CDCl_3$ or CD_3OD as solvent (0.6 mL). Mass spectral analyses were performed on UltrOTOF-Q (Bruker Daltonics, Billerica, MA, USA) in the cationized ion region. The heated capillary and voltage were maintained at 250 °C and 3 kV, respectively. A 20 V cone energy for ion extraction and mass spectrometry data were acquired at positive and negative modes for all compounds. High performance liquid chromatography (HPLC) was carried out in a semi-preparative LC-8A Shimadzu system with SPD-10AV Shimadzu UV detector (Tokyo, Japan); using a Phenomenex Gemini C18 column (250 × 10 mm, 5 μm), isocratic system of 50% acetonitrile-water and 35% acetonitrile-water, and a flow rate 4.7 mL min^{-1}. Detection was performed at 254 and 282 nm. All solvents were filtered through a 0.45 mm nylon membrane filter prior to analysis. Open column chromatography was run using silica gel 60 (70-230 mesh, Maherey-Nagel). Thin layer chromatography (TLC) was performed on precoated silica gel aluminium sheets (Maherey-Nagel) by detection with a spraying reagent (vanillin/sulfuric acid/EtOH solution) followed by heating at 100 °C and with reagent NP-PEG (diphenylborinic acid aminoethylester-polyethylene glycol) for flavonoid detection.

Plant material

Leaves of *Vatairea guianensis* were collected in November 2010 from Belém, State of Pará, Brazil. Identification was made by Manoel R. Cordeiro from Embrapa Amazônia Oriental, Pará, Brazil and a voucher specimen (IAN – 187050) has been deposited in the herbarium of Embrapa Amazônia Oriental.

Extraction and isolation

Dried and powdered leaves of *Vatairea guianensis* (1.0 kg) were extracted with ethanol by maceration at room temperature. The solvent was removed under vacuum, furnishing a residue (180.0 g). The crude ethanol residue (50.0 g) was dissolved in 500 mL MeOH-H_2O mixture (9:1), then partitioned three times with *n*-hexane (3 × 500 mL), ethyl acetate (3 × 500 mL) and the remaining hydroalcoholic phase. The extracts obtained were dried to provide three fractions: an *n*-hexane fraction (7.5 g), an EtOAc fraction (22.0 g) and a remaining MeOH-H_2O fraction (20.0 g). The *n*-hexane fraction (7.0 g) was subjected on silica gel column chromatography with gradient elution of *n*-hexane-EtOAc (9:1, 8:2, 7:3, 5:5 and 0:10) and EtOAc-MeOH (5:5 and 0:10), to obtain seven fractions (Fr.1–Fr.7), respectively. In fraction Fr-1 (1.1 g) eluted with *n*-hexane/EtOAc (9:1), after analysis by TLC and ^{1}H and ^{13}C NMR, it was possible to identify a mixture of pentacyclic triterpenes (**5**-**9**). The fraction Fr-2 (0.5 g) eluted with *n*-hexane/EtOAc (8:2) was sonicated in 4.8 mL of acetonitrile for 1 min. Next, 1.2 mL of H_2O was added and sonicated again for 1 min. The solution was subjected to solid phase extraction (SPE) in a C18 cartridge (Phenomenex, 1 g of stationary phase / 6 mL). After evaporation, the residue (about 175 mg) was submitted to semi-preparative reversed phase HPLC (250 × 10 mm Phenomenex Gemini C18, 50% acetonitrile-water, flow rate 4.7 mL min^{-1}, 254 nm) to yield **1** (25.0 mg), **2** (16.0 mg) and **4** (15.0 mg), which showed chromatographic peak retention times of 35.0, 39.0 and 51.0 min, respectively. The fraction Fr-6 (0.6 g) eluted with EtOAc-MeOH (5:5), after clean-

up (treated by ultrasonic bath using 4.8 mL of acetonitrile for 1 min, after 1.2 mL of H_2O was added and sonicated again for 1 min, after which the solution was subjected to solid phase extraction (SPE) in a C18 cartridge). A residue (90.0 mg) was obtained that was submitted to semi-preparative reversed phase HPLC (250 × 10 mm Phenomenex Gemini C18, 35% acetonitrile-water, flow rate 4.7 mL min^{-1}, 282 nm) to yield **3** (15.0 mg), which showed chromatographic peak retention time of 8.3 min. These compounds were identified by NMR and mass spectrometry methods, and comparison with available reported data.

DPPH assays

A stock solution of DPPH• radical (0.5 mmol L^{-1}) in methanol was prepared. The solution was diluted in methanol (60 μmol L^{-1} approx.) measuring an initial absorbance of 0.62 ± 0.02 in 517 nm at room temperature. The reaction mixture was composed by 1950 μL of DPPH• solution and 50 μL of the samples diluted in different methanol portions. The final concentrations were of 10.0, 7.5, 5.0 and 2.5 μg mL^{-1} for the crude extract, 75.0, 50.0, 25.0 and 12.5 μg mL^{-1} for substances **2** and **3** and 8.0, 6.0, 4.0 and 2.0 μg mL^{-1} for the Trolox standard. For each sample a methanol blank was also measured. The absorbance was measured at 517 nm during 60 min. All experiments were conducted in triplicate and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard antioxidant. The radical scavenging activity of each sample was calculated by the DPPH• inhibition percentage ($\%I_{DPPH}$) according to equation 1, where A and B are the blank and sample absorbance values in the end reaction.[9]

$$\%I_{DPPH} = \left(\frac{A-B}{A}\right)100 \quad (1)$$

The concentration of antioxidant required for 50% scavenging of DPPH• radicals (IC_{50}) was determined by linear regression using Windows/Excel. All analyses were performed in triplicate. The data were expressed as means ± standard deviation (SD) and analyzed using GraphPad (version 5.0). One-way analysis of variance (ANOVA) and Tukey multiple comparisons were performed in order to test any significant differences between the means. Differences between means at the 5% level were considered significant.

5,3'-Dihydroxy-4'-methoxy-2",2"-dimethylpyrano [5",6":8,7] isoflavone (**1**)

Amorphous pale green solid; UV λ_{max}/nm (acetonitrile-water): 268, 304 (*sh*); HRESITOF-MS *m/z* 367.11901 $[M + H]^+$ (calc. for $C_{21}H_{19}O_6^+$, 367,11816); ^1H and ^{13}C NMR spectral data: see Tables 1 and 2.

5,7-Dihydroxy-3',4'-methylenedioxy-8-prenyl-isoflavone (**2**)

Yellow amorphous powder; UV λ_{max}/nm (acetonitrile-water): 265, 292 (*sh*); HRESITOF-MS *m/z* 367.11723 $[M + H]^+$ (calc. for $C_{21}H_{19}O_6^+$, 367.11816); ^1H and ^{13}C NMR spectral data: see Tables 1 and 2.

5,3'-Dihydroxy-4'-methoxy-7-*O*-β-glucopyranoside-8-prenyl-isoflavone (**3**)

Amorphous reddish solid; UV λ_{max}/nm (acetonitrile-water): 265, 290 (*sh*); HRESITOF-MS *m/z* 531.18615 $[M + H]^+$(calc. for $C_{27}H_{31}O_{11}^+$, 531.18664); ^1H and ^{13}C NMR spectral data: see Tables 1 and 2.

Results and Discussion

The hexane fraction from the ethanol extract of *V. guianensis* leaves was fractionated as indicated in the Experimental section leading to the isolation of new isoflavones **1**-**3**, together with known compounds derrone (**4**), lupeol, α-amirin, β-amirin, germanicol and betulin acid, which were identified by comparison of their spectral data with those reported in literature.[10,11]

Compound **1** was obtained as an amorphous pale green solid, with the molecular formula $C_{21}H_{18}O_6$, based on the $[M+H]^+$ peak at *m/z* 367.11901 (calc. for $C_{21}H_{19}O_6^+$, 367.11816) in the HRESITOF-MS, and confirmed by ^1H and ^{13}C NMR experiments (Tables 1 and 2, respectively). The ^1H NMR signals at δ_H 7.87 (H-2) and ^{13}C NMR signal at δ_C 152.5 (C-2), 123.7 (C-3) and 180.8 (C-4), were typical of isoflavones.[12] Additionally, the ^1H NMR spectrum exhibited signals in the aromatic region at δ_H 6.91 (d, 1H, *J* 8.1 Hz), 7.03 (brd, 1H, *J* 8.1 Hz) and 7.06 (brs, 1H), which indicated an ABX spin system of a 1,3,4-trisubstituted phenyl group, as well as one singlet at δ_H 6.28 assigned to a pentasubstituted benzene ring. The signals observed at δ_H 5.58 and 6.67 (d, 1H each, *J* 9.9 Hz) and 1.47 (s, 6H) revealed a 2,2-dimethylchromene ring attached to an aromatic ring, whereas the singlets at δ_H 12.95 and 3.91 indicated the presence of a hydroxyl chelated to carbonyl and one OMe group connected to an aromatic ring, respectively. All couplings were confirmed through analysis of the ^1H-^1H COSY spectrum. Besides the signals related to C-ring carbons, the ^{13}C NMR spectrum of **1** exhibited another 17 signals attributed to eighteen carbons with aid of the HETCOR and HMBC (Table 2) experiments. The 2,2-dimethylchromene ring attached to

A-ring at C-7 and C-8 was deduced by $^{3}J_{C,H}$ correlations from H-2 (δ_H 7.87) and H-4'' (δ_H 6.67) to C-8a (δ_C 152.1) observed in the HMBC spectrum (Table 2), as well as $^{2}J_{C,H}$ correlation between H-6 (δ_H 6.28) and OH-5 (δ_H 12.95) with C-5 (δ_C 162.2). The location of the OMe and OH groups at C-4' and C-3' of the aromatic B-ring, respectively, was supported by the $^{3}J_{C,H}$ correlations from H-6' (δ_H 7.03) and OMe-4' (δ_H 3.91) to oxidized aromatic carbon C-4' (δ_C 146.8) and confirmed by the NOE effect observed in the NOE difference experiment, which revealed spatial interactions between H-5' (δ_H 6.91) and OMe-4' (δ_H 3.91). From the above features we can establish that compound **1** is 5,3'-dihydroxy-4'-methoxy-2'',2''-dimethylpyrano-(5'',6'':8,7)-isoflavone, here isolated for the first time from a natural source, though it has already been produced by synthesis.[13] Full spectrometric data for this compound are presented.

Compound **2** was obtained as a yellow amorphous powder and its molecular formula was defined as $C_{21}H_{18}O_6$ on the basis of the quasi-molecular ion $[M+1]^+$ observed at *m/z* 367.11723 (calc. for $C_{21}H_{19}O_6^+$, 367.11816) in HRESITOF mass spectrometry analysis. The ^{1}H NMR signal at δ_H 7.91 (H-2) and ^{13}C NMR signal at δ_C 152.8 (C-2) were characteristic of the isoflavone skeleton, similarly to compound **1**. Additionally, the ^{1}H NMR spectrum (Table 1) exhibited signals at δ_H 6.86 (d, *J* 7.8 Hz, H-5'), 6.94 (dd, *J* 7.8 and 1.5 Hz, H-6') and 7.03 (d, *J* 1.5 Hz, H-2'), which indicated an AMX spin system of a 1,3,4-trisubstituted B-ring, as well as three singlets at δ_H 6.29 (1H), attributed to a pentasubstituted A-ring, δ_H 5.99 (2H) assigned to a methylenedioxy unit and δ_H 12.81 related to a hydroxyl group bonded to C-5. The ^{13}C NMR spectrum of **2** (Table 2) exhibited 21 signals attributed to twenty-one carbons with the aid of the HETCOR and HMBC experiments. The C-prenyl group [δ_H 3.46 (d, *J* 6.9 Hz, H-1''), 5.22 (brt, *J* 6.9 Hz, H-2''), 1.74 (s, Me-*trans*) and 1.82 (s, Me-*cis*)] attached at C-8 was deduced from the $^{3}J_{C,H}$ correlations in the HMBC experiments (Table 2) of the signals at δ_H 7.91 (H-2) and 3.46 (H-1') with the signal at δ_C 155.0 (C-8a). On the other hand, the HMBC $^{2,3}J_{C,H}$ correlations from δ_H 7.02 (H-2') and 6.86 (H-5') to δ_C 147.8 and 147.7 (C-3'/C-4') revealed the connection of a methylenedioxy unit to C-3' and C-4'. The structure of **2** was then identified as 5,7-dihydroxy-3',4'-methylenedioxy-8-prenyl-isoflavone. This compound is a new natural product, although it has been cited as a synthetic product.[14] Its spectrometric data are presented for the first time.

Compound **3**, an amorphous reddish solid, showed molecular formula $C_{27}H_{30}O_{11}$ that was established by the quasi-molecular ion at *m/z* 531.18615 $[M+H]^+$ in the HRESITOF-MS (calc. for $C_{27}H_{31}O_{11}^+$, 531.18664), and confirmed by ^{1}H and ^{13}C NMR experiments (Tables 1 and 2). Similarly to the compounds **1** and **2**, **3** is an isoflavone based on the ^{1}H NMR signal at δ_H 8.20 (H-2) and the ^{13}C NMR signal at δ_C 155.5 (C-2). Additionally, the ^{1}H NMR spectrum (Table 1) displayed signals in the aromatic region at δ_H 7.06 (d, 1H, *J* 1.8 Hz,) and 6.78-6.99 (m, 2H), which can be attributed, alike to compound **1**, to the ABX spin system of a 1,3,4-trisubstituted B-ring. The pentasubstituted A-ring has been established as in compounds **1** and **2**, based on the singlet signal at δ_H 6.64 (s, 1H). In the ^{1}H NMR spectrum of **3** signals were also observed for a OMe group at δ_H 3.88 (s, 3H), a C-prenyl group at δ_H 3.60 (d, *J* 7.2 Hz, 2H-1''), 5.22 (*t*, *J* 7.2 Hz, H-2''), 1.82 (s, 3H-4'') and 1.66 (s, 3H-5''), and a glucose unit at δ_H 5.05 (d, *J* 7.2 Hz, H-1'') and 3.41-3.75 (m, H-2''- H-6''). The glucose residue was identified by comparison of NMR data with the literature,[15] where the anomeric proton signal at δ_H 5.05 with a large coupling constant of 7.2 Hz implies that the glucose moiety must have a β-glucopyranose form.[16] All the couplings were confirmed through analysis of the ^{1}H-^{1}H COSY spectrum. In addition to signals related to the isoflavone skeleton containing a carbonyl (δ_C 182.7) chelated to OH-5 group,[17,18] the ^{13}C NMR spectrum of **3** exhibited another 12 signals attributed with the aid of the

Table 1. ^{1}H NMR Chemical Shifts (δ_H / ppm) of Compounds **1**, **2** (in $CDCl_3$) and **3** (in CD_3OD)[a]

H	1	2	3
	δ_H	δ_H	δ_H
2	7.87 (s)	7.91 (s)	8.20 (s)
6	6.28 (s)	6.29 (s)	6.64 (s)
2'	7.06 (brs)	7.03 (d, 1.5)	7.06 (d, 1.8)
5'	6.91 (d, 8.1)	6.86 (d, 7.8)	6.78-6.99 (m)
6'	7.03 (brd, 8.1)	6.94 (dd, 1.5 and 7.8)	6.78-6.99 (m)
1''		3.46 (d, 6.9)	3.60 (d, 7.2)
2''		5.22 (brt, 6.9)	5.22 (brt, 7.2)
3''	5.58 (d, 9.9)		
4''	6.67 (d, 9.9)		
2Me-2''	1.47 (s)		
4'' (Me, *cis*)		1.82 (s)	1.82 (s)
5'' (Me, *trans*)		1.74 (s)	1.66 (s)
OMe-4'	3.91 (s)		3.88 (s)
OCH_2O		5.99 (s)	
OH-5	12.95 (s)	12.81 (s)	
1''' (Glc)			5.05 (d, 7.2)
2'''- 6''' (Glc)			3.41-3.75 (m)

[a]^{1}H NMR data were recorded at 300 MHz. Multiplicity and coupling constants (*J* / Hz) are in parenthesis.

HETCOR and HMBC (Table 2) experiments to the carbons of OMe and prenyl groups, as well as a glucose unit (δ_C 101.8, 78.3, 78.2, 74.9, 71.1, 62.3). The C-8 location of the prenyl group was supported by the $^3J_{C,H}$ correlations in the HMBC spectrum from the signals at δ_H 8.20 (H-2) and 3.60 (H-1'') to δ_C 156.0 (C-8a). Moreover, the cross-peaks in the HMBC spectrum between H-6 (δ_H 6.64) and H-1''' (δ_H 5.05) of the glucose unit with C-7 (δ_C 162.1) indicated that the glucose residue was attached to the 7-hydroxyl of the isoflavone moiety. The location of the OMe and OH groups at C-4' and C-3' of the B-ring, respectively, was sustained by combining the substitution pattern on the aromatic B-ring (1,3,4-trisubstituted) displayed in the ^{1}H NMR spectrum with the NOE effects observed in the NOE difference spectra, which revealed spatial interactions between H-5' and OMe-4'. Therefore, **3** was characterized as 5,3'-dihydroxy-4'-methoxy-7-*O*-β-glucopyranoside-8-prenyl-isoflavone, a new isoflavone glucoside.

The crude extract of leaves showed strong radical scavenging capacity, with inhibition percent of 77.2 at 22.0% at concentrations of 10.0 at 2.5 μg mL^{-1}, respectively, and IC_{50} value of 6.2 ± 0.4 μg mL^{-1} (Trolox, IC_{50} = 4.4 ± 0.1 μg mL^{-1}). The results obtained from isolated isoflavones **1**-**3** showed that all tested samples

Table 2. ^{13}C NMR Chemical Shifts (δ_C / ppm) of Compounds **1**, **2** (in $CDCl_3$) and **3** (in CD_3OD)[a]

C	1		2		3	
	δ_C	HMBC[c]	δ_C	HMBC[c]	δ_C	HMBC[c]
2	152.5		152.8		155.5	
3	123.7[b]	2, 2', 6'	123.3	2	125.0[b]	2
4	180.8	2	181.1	2	182.7	2
4a	106.0	6, OH-5	105.4	6	107.8	6
5	162.2	6, OH-5	160.5	6	162.0	6
6	100.3	OH-5	99.6		99.7	
7	159.5	6, 4'	160.8	6, 1'	162.1	6, 1"
8	101.1	6, 3', 4'	106.0	6, 1'	110.4	6, 1'
8a	152.1	2, 4'	155.0	2, 1'	156.0	2, 1'
1'	123.5[b]	2, 2', 5', 6'	124.3	5'	124.4[b]	2', 5', 6'
2'	115.0	6'	109.6	6'	117.3	6'
3'	145.6	2', 5'	147.8[b]	2', 5'	147.4	
4'	146.8	2', 5', 6', OMe-4'	147.7[b]	2', 5'	149.3	2', OMe-4'
5'	110.7		108.5		112.6	
6'	120.9	2'	122.4		121.6	2'
1"			21.5		22.5	
2"	78.0	3', 4', 2Me-2'	121.1	1", 4", 5"	123.6	1", 4", 5"
3"	127.4	2Me-2'	134.7	1", 4", 5"	132.6	1", 4", 5"
4" (Me, *cis*)	114.5		17.9		18.0	5"
5" (Me, *trans*)			25.8		25.9	4"
2Me-2'	28.2					
OMe-4'	56.0				56.4	
OCH_2O			101.2			
1''' (Glc)					101.8	
2''' (Glc)					74.9	
3''' (Glc)					78.2	
4''' (Glc)					71.1	
5''' (Glc)					78.3	
6''' (Glc)					62.3	

[a]^{13}C NMR data were recorded at 75 MHz; number of hydrogens bound to carbon atoms deduced by comparative analysis of ^{1}H and DEPT ^{13}C NMR spectra; [b]the values can be exchanged; [c]^{1}H-^{13}C HMBC correlations are from the carbon(s) specified to the protons indicated.

were capable of scavenging free radical DPPH•, however, compound **1** was weakly active with an inhibition percentage below 50.0% at 100 μg mL^{-1}. Compounds **2** and **3** presented greater inhibition effects, with inhibition percentage above 50.0% during 60 min of reaction at 25 and 50 μg mL^{-1} concentrations, respectively. However, compounds **2** and **3** were about 6 and 14 times less active than Trolox with IC_{50} values of 29.5 ± 2.5 μg mL^{-1} and 64.3 ± 2.6 μg mL^{-1}, respectively.

The free radical scavenging activity of flavonoids and other phenols is mostly due to their aromatic hydroxyl groups, which afford greater stability to the phenolic radical as soon as it is formed, after one hydrogen radical donation to DPPH•.[19] Although compounds **1**-**3** possess two phenolic hydroxyl groups each, the isoflavonoid **2** was more effective in promoting DPPH• reduction, when compared to **1** and **3**. Early studies showed that the presence of a substituent (sugar residues) in position C-7 of A-ring in the flavonoids reduces the sequestering activity of the radicals, since the flavonoid structure loses its coplanarity due to the presence of voluminous groups,[20,21] and methylation in hydroxyl group in the *para*-position decreased DPPH• scavenging activity.[22] On the other hand, the methylenedioxy function contributes to stabilization of the phenoxy radical.[23] The fact that much higher values of IC_{50} were found for the isolated compounds when compared to Trolox suggests that the significant anti-radical activity exhibited by the ethanolic extract of *V. guianensis* leaves may be attributed to the effect of synergism in the substances present in this extract.

Conclusions

Phytochemical investigation from the leaves of *Vatairea guianensis* (Fabaceae) resulted in the isolation of compounds belonging to the isoflavone and triterpene classes, and it should be noted that the isoflavone class is being cited for the first time for this species. The evaluation of antioxidant activity, based on the method of sequestering radical DPPH• demonstrated that the ethanolic extract of *V. guianensis* leaves presents a high power for scavenging radical DPPH•, similar to the TROLOX standard, while the isoflavones tested present low anti-radical reaction, which suggests that the greatest antioxidant potential of the extract may be associated with synergism among its components.

Supplementary Information

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

Acknowledgments

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Supplementary Information

New Isoflavones from the Leaves of *Vatairea guianensis* Aublé

Ronilson Freitas de Souza,[a] Victor H. S. Marinho,[a] Geilson A. da Silva,[a] Livio M. Costa-Jr.,[b] Joyce Kelly R. da Silva,[c] Gilmara N. T. Bastos,[d] Alberto C. Arruda,[a] Milton N. da Silva[a] and Mara Silvia P. Arruda*[,a]

[a]Programa de Pós-Graduação em Química, Instituto de Ciências Exatas e Naturais, Universidade Federal do Pará, Campus Universitário do Guamá, 66075-970 Belém-PA, Brazil

[b]Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão, MA-230, km 04, s/n, Boa Vista, 65500-000 Chapadinha-MA, Brazil

[c]Programa de Pós-Graduação em Biotecnologia and [d]Programa de Pós-Graduação em Neurociências e Biologia Celular, Instituto de Ciências Biológicas, Universidade Federal do Pará, Campus Universitário do Guamá, 66075-970 Belém-PA, Brazil

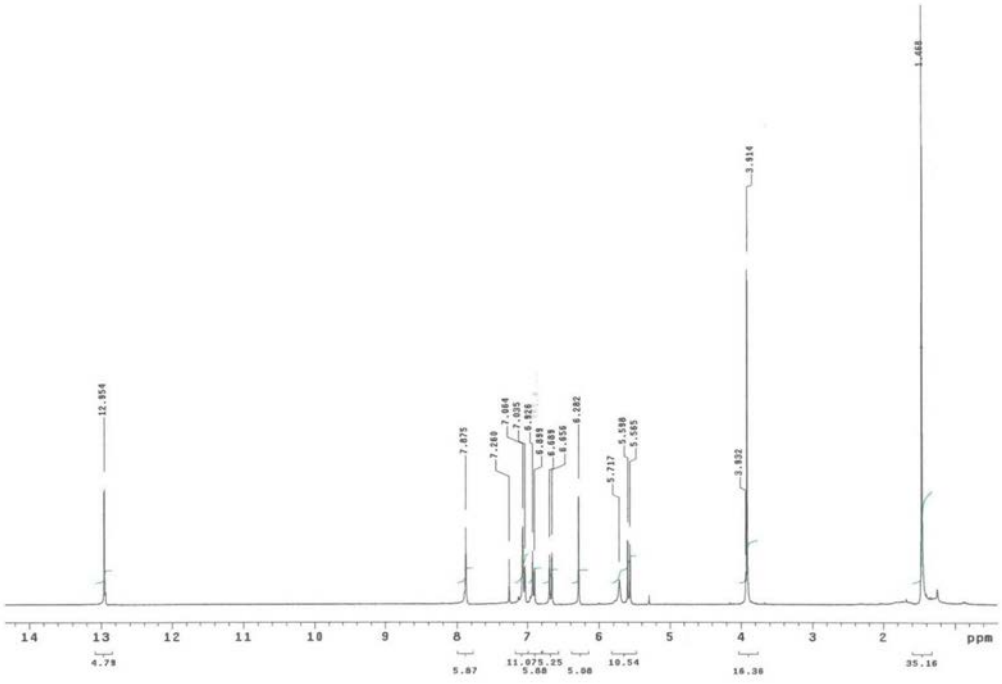

Figure S1. ^{1}H NMR spectrum of **1** ($CDCl_3$, 300 MHz).

*e-mail: mspa@ufpa.br

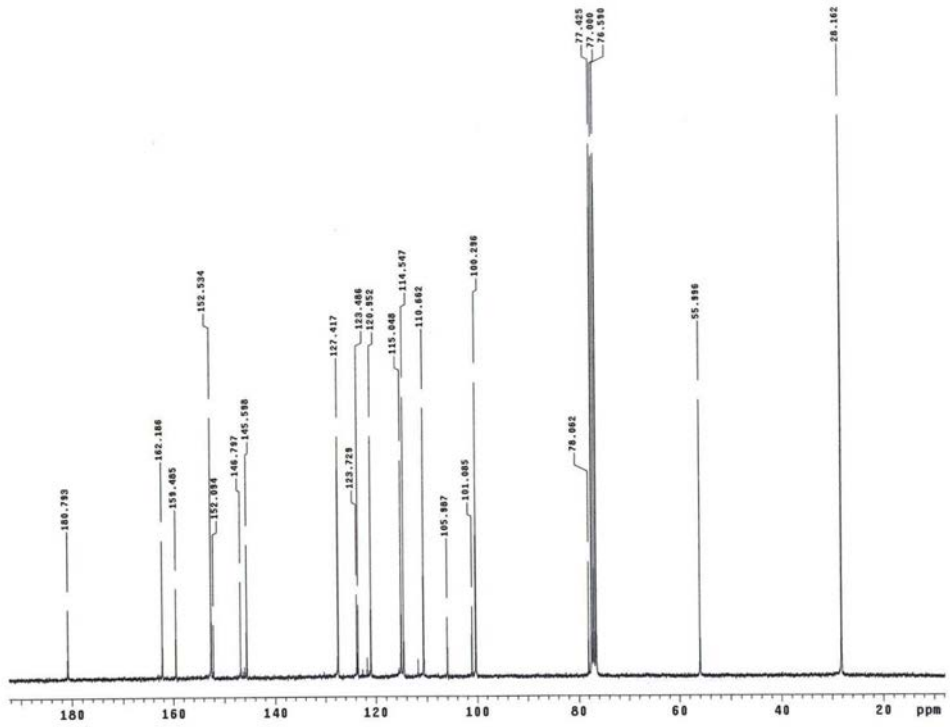

Figure S2. ^{13}C NMR spectrum of **1** ($CDCl_3$, 75 MHz).

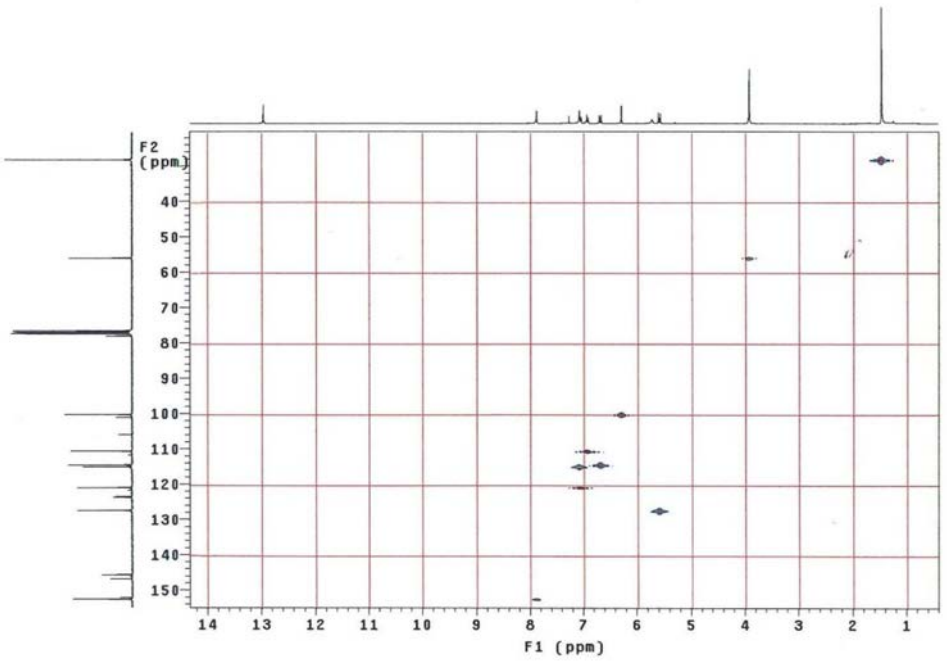

Figure S3. HETCOR NMR spectrum of **1** ($CDCl_3$, 300 x 75 MHz).

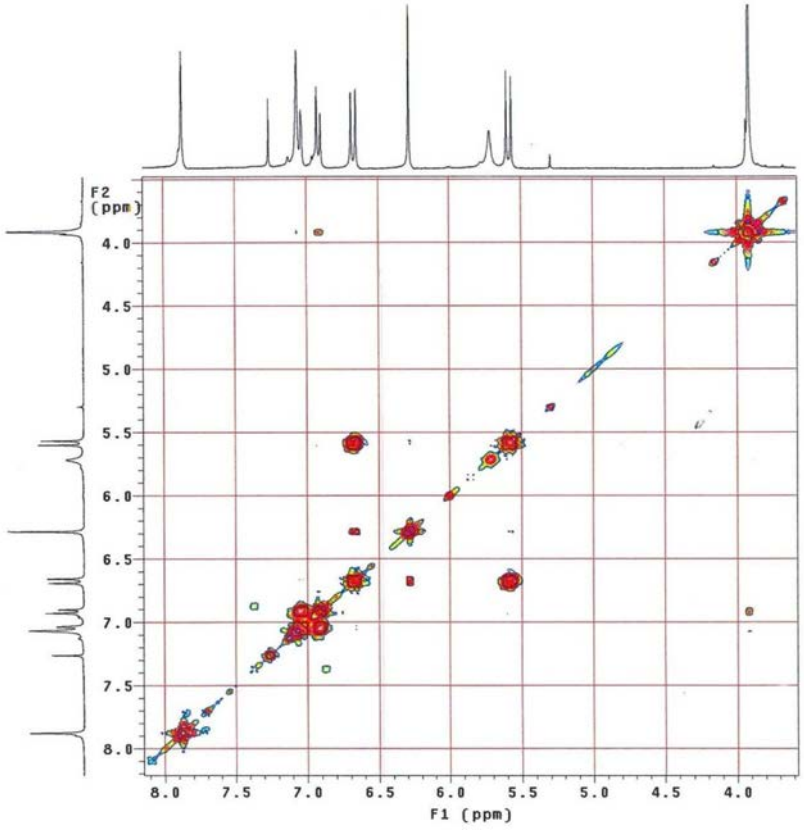

Figure S4. COSY NMR spectrum of **1** ($CDCl_3$, 300 MHz).

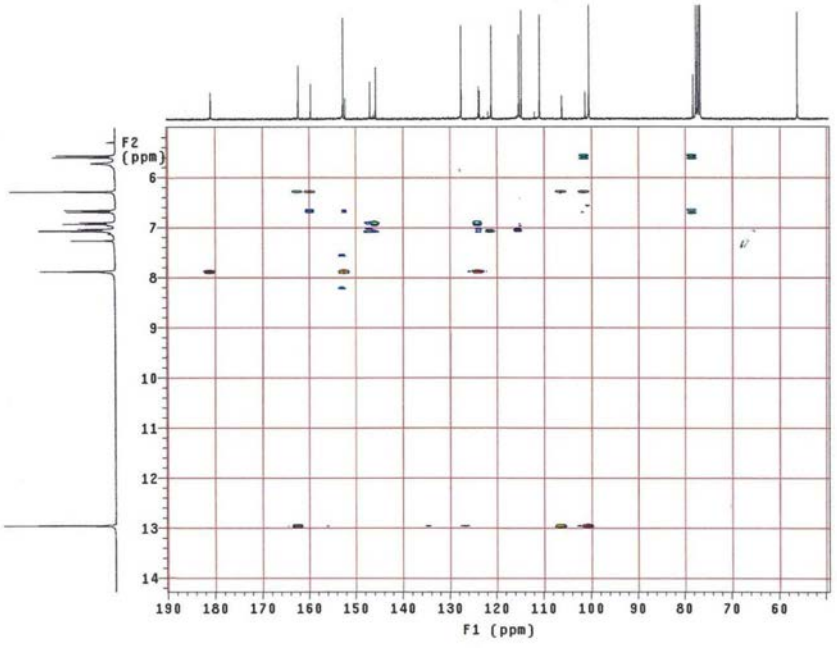

Figure S5. HMBC NMR spectrum of **1** ($CDCl_3$, 300 x 75 MHz) (expansion 1).

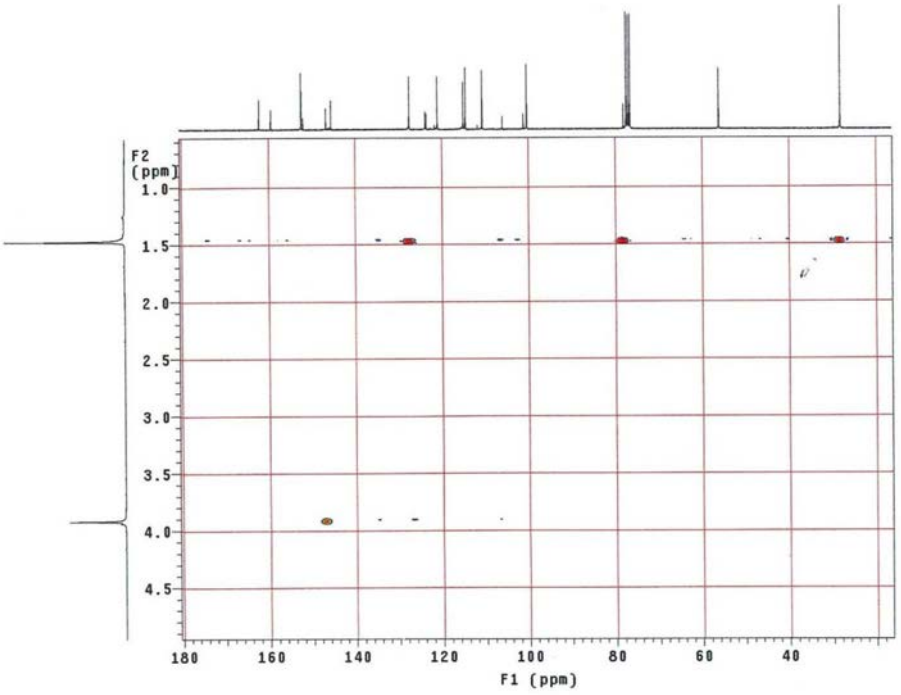

Figure S6. HMBC NMR spectrum of **1** ($CDCl_3$, 300 x 75 MHz) (expansion 2).

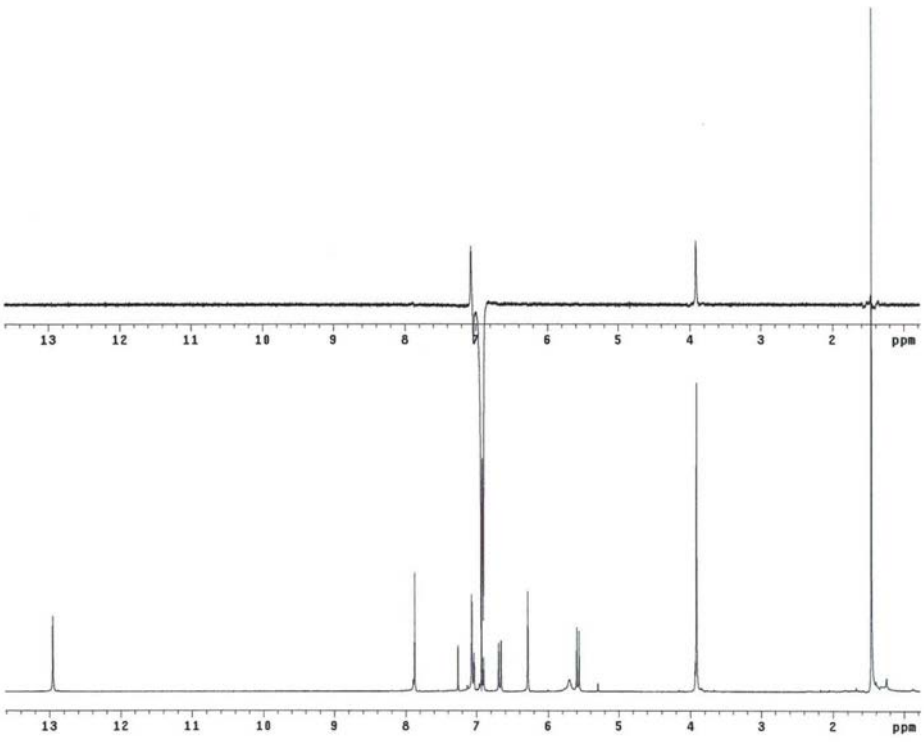

Figure S7. NOEdiff NMR spectrum of **1** ($CDCl_3$, 75 MHz)**.**

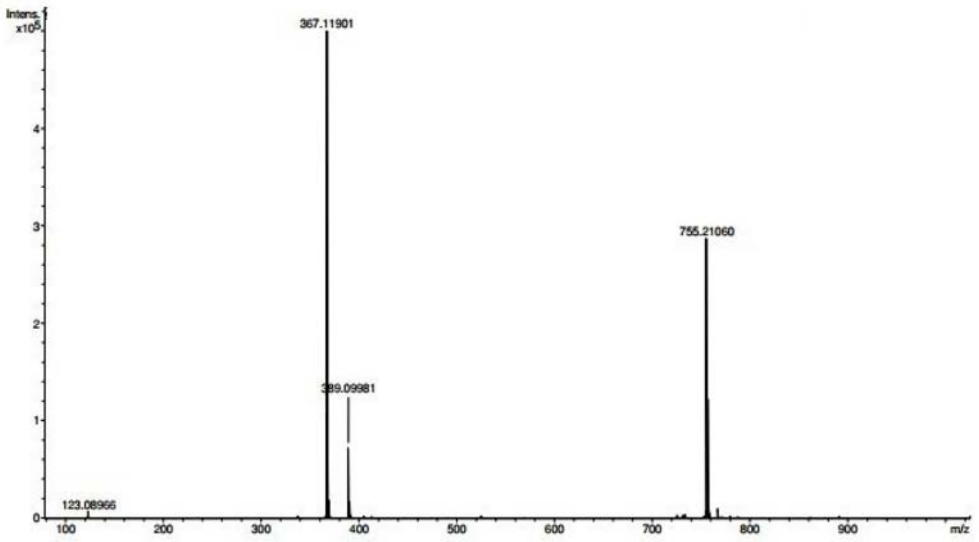

Figure S8. HRESITOF-MS spectrum of **1.**

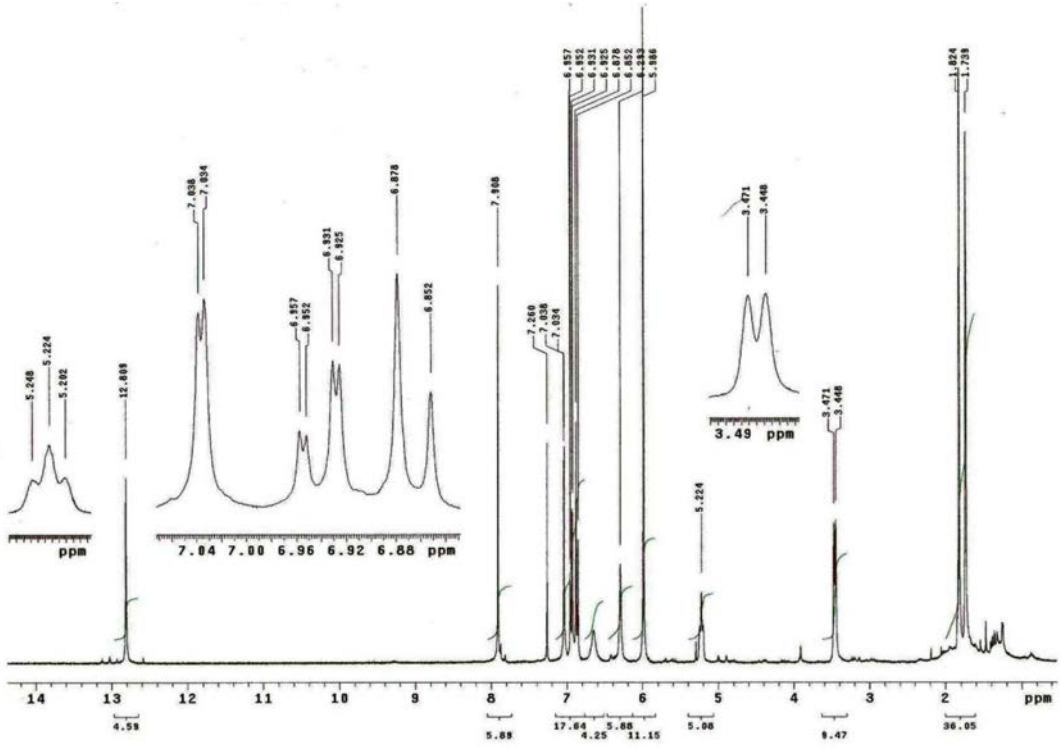

Figure S9. ^{1}H NMR spectrum of **2** ($CDCl_3$, 300 MHz).

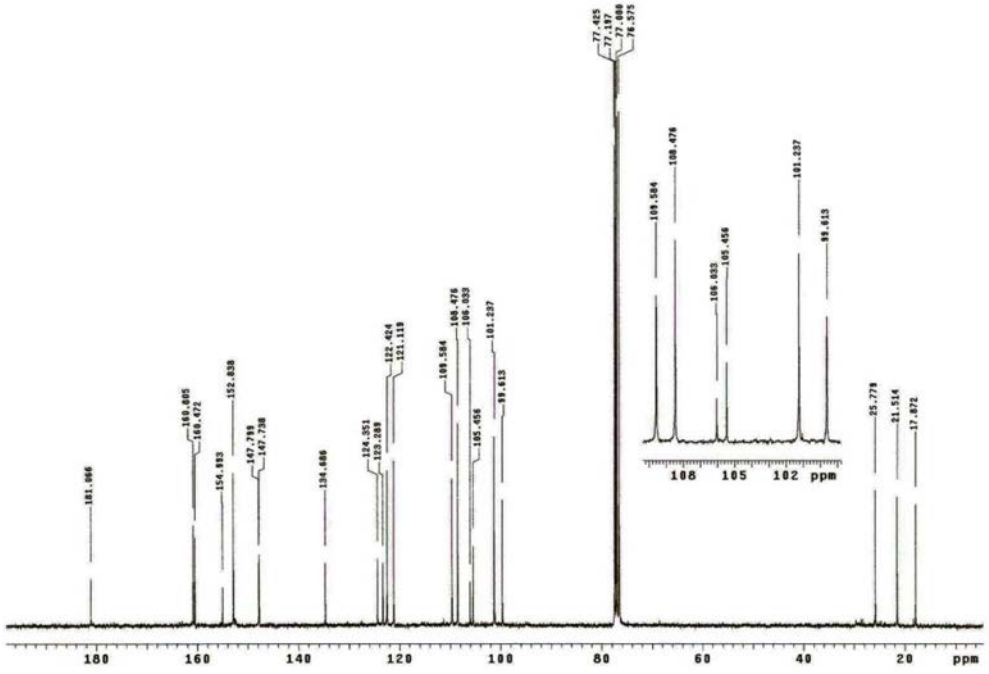

Figure S10. ^{13}C NMR spectrum of **2** ($CDCl_3$, 75 MHz).

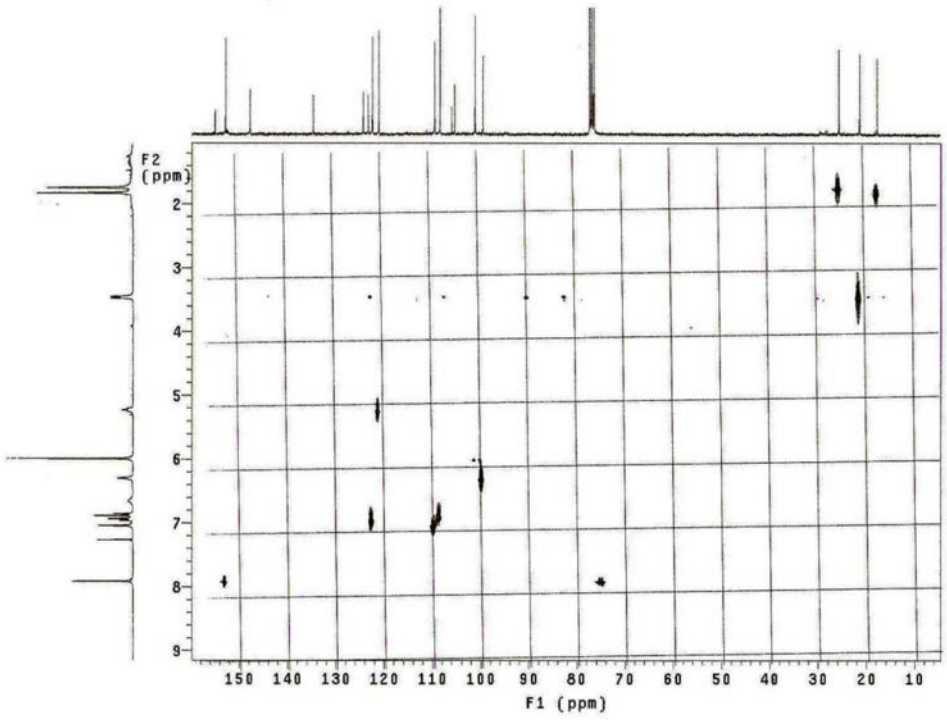

Figure S11. HETCOR NMR spectrum of **2** ($CDCl_3$, 300 x 75 MHz).

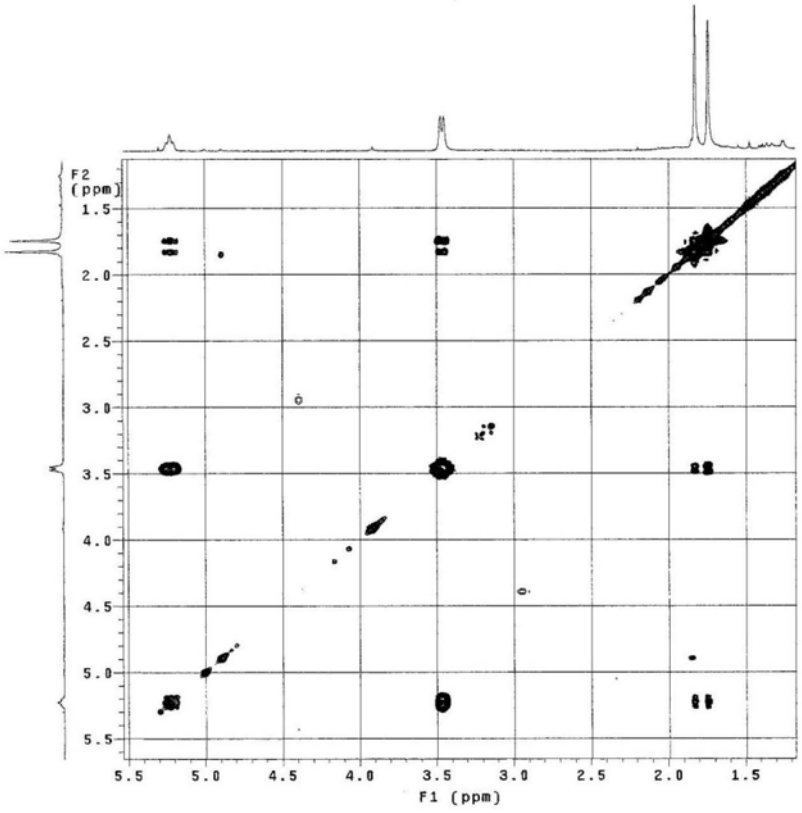

Figure S12. COSY NMR spectrum of **2** ($CDCl_3$, 300 MHz).

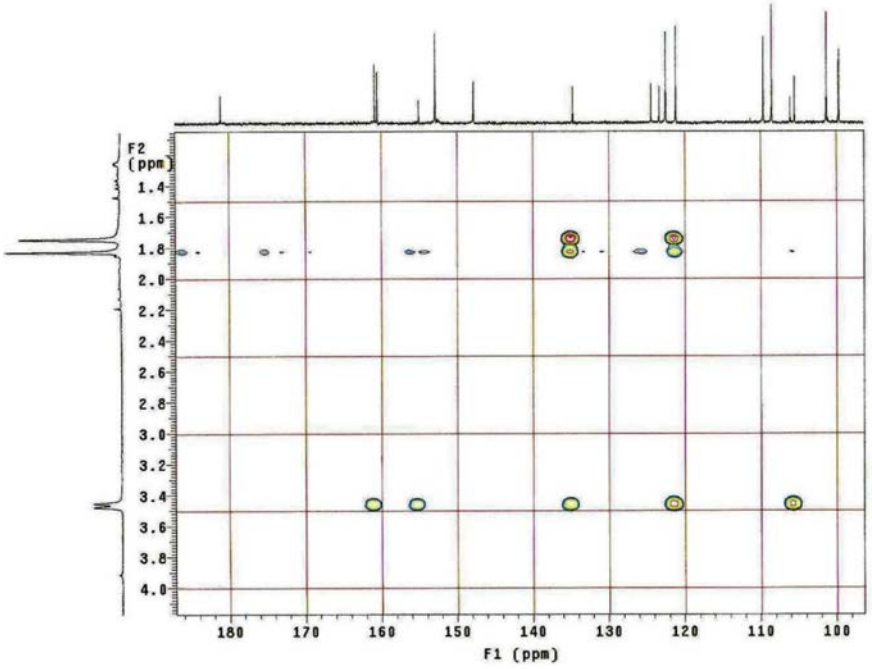

Figure S13. HMBC NMR spectrum of **2** ($CDCl_3$, 300 x 75 MHz) (expansion 1).

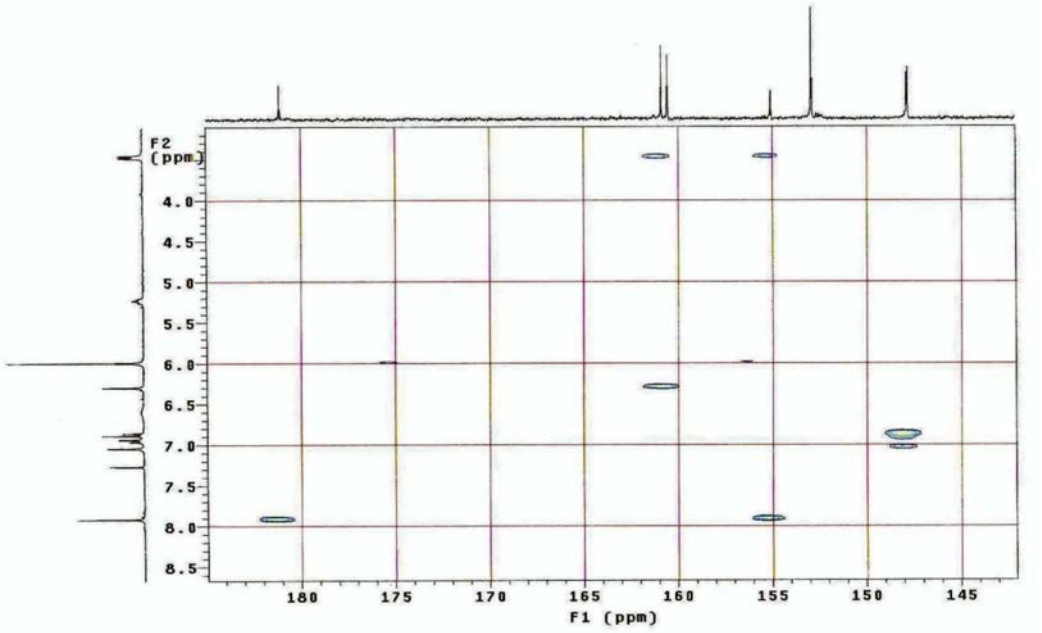

Figure S14. HMBC NMR spectrum of **2** ($CDCl_3$, 300 x 75 MHz) (expansion 2).

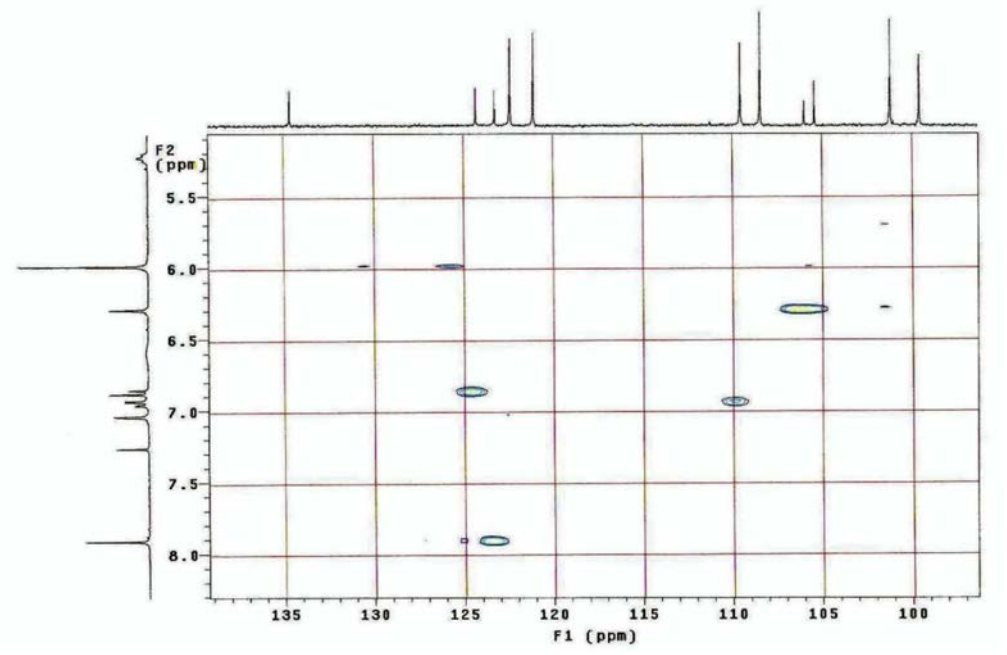

Figure S15. HMBC NMR spectrum of **2** ($CDCl_3$, 300 x 75 MHz) (expansion 3).

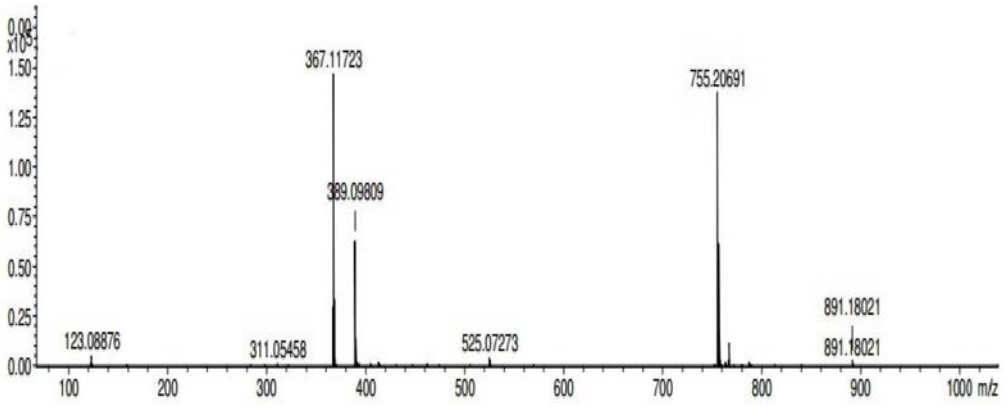

Figure S16. HRESITOF-MS spectrum of **2.**

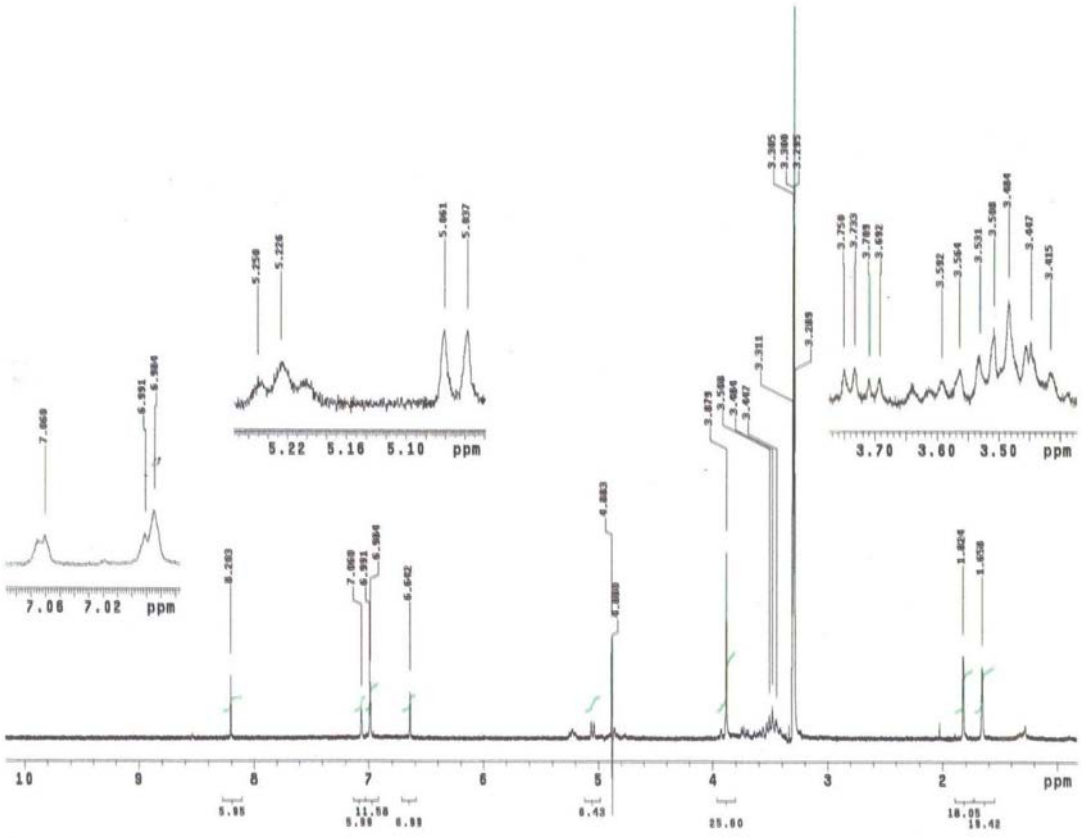

Figure S17. ^{1}H NMR spectrum of **3** (CD_3OD, 300 MHz).

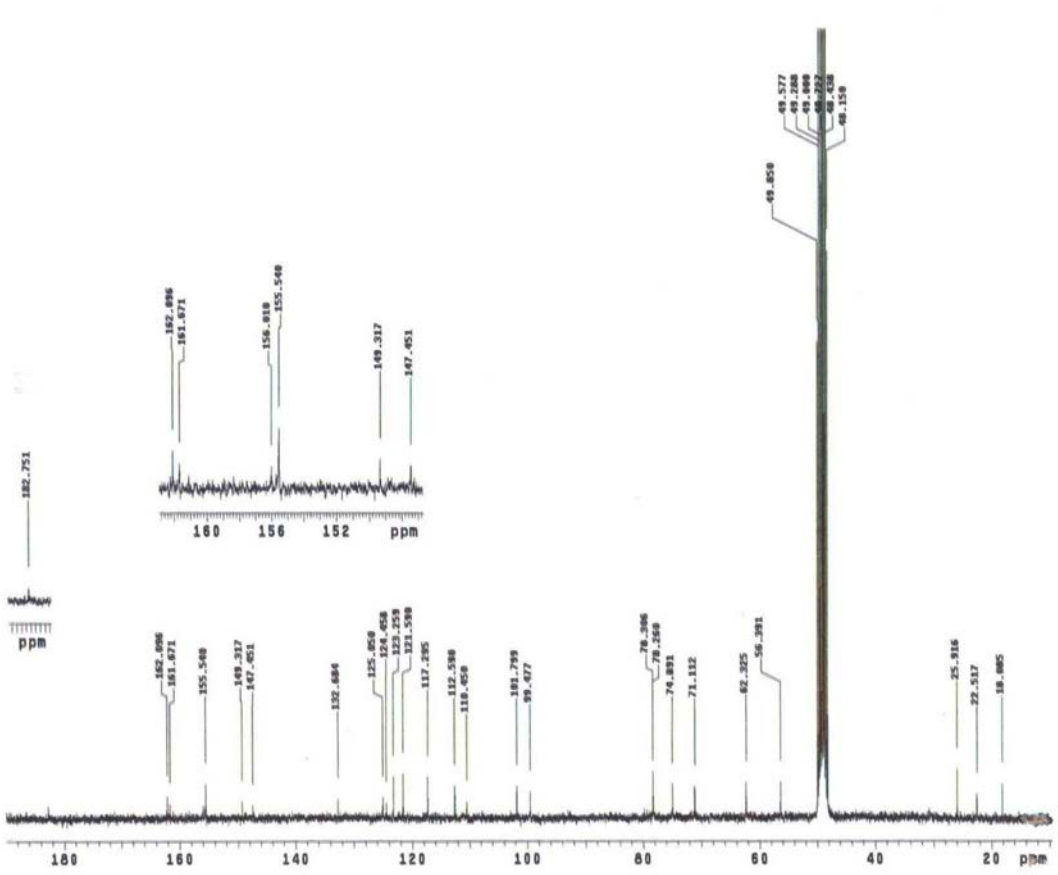

Figure S18. ^{13}C NMR spectrum of **3** (CD_3OD, 75 MHz).

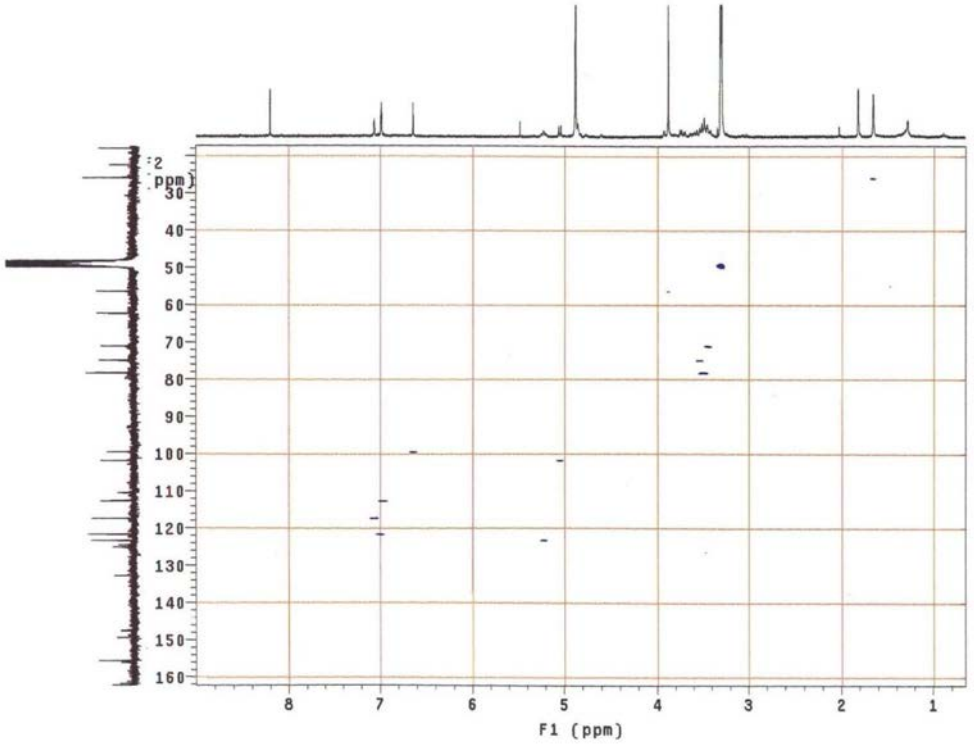

Figure S19. HETCOR NMR spectrum of **3** (CD_3OD, 300 x 75 MHz).

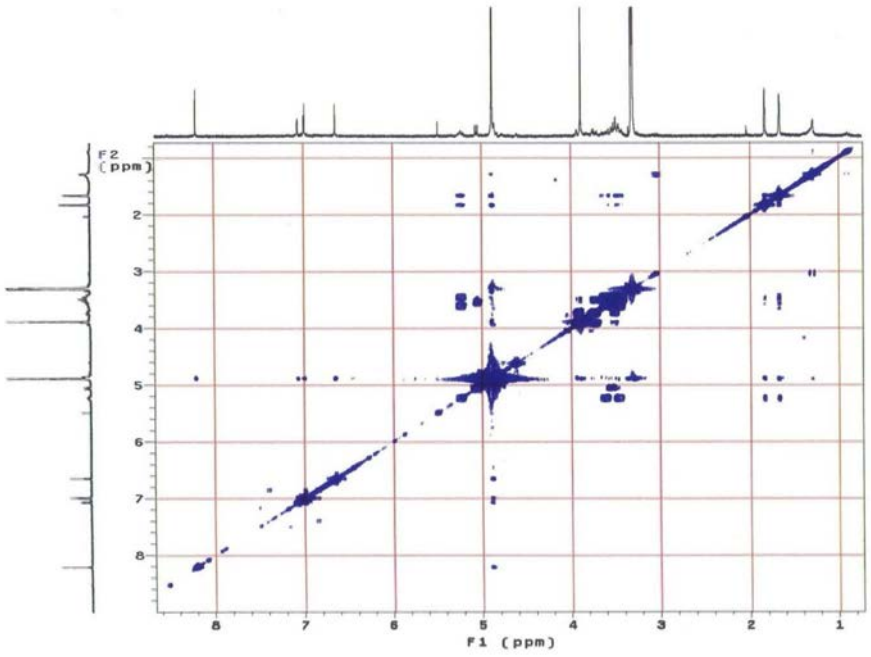

Figure S20. COSY NMR spectrum of **3** (CD_3OD, 300 MHz).

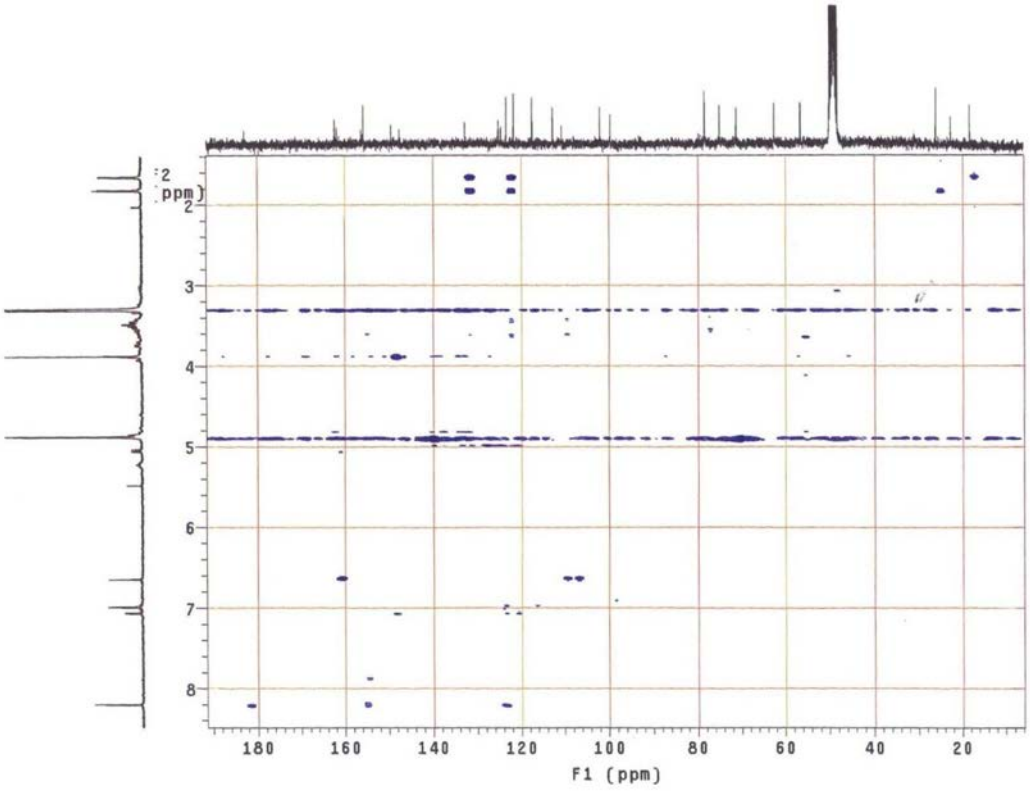

Figure S21. HMBC NMR spectrum of **3** (CD_3OD, 300 x 75 MHz).

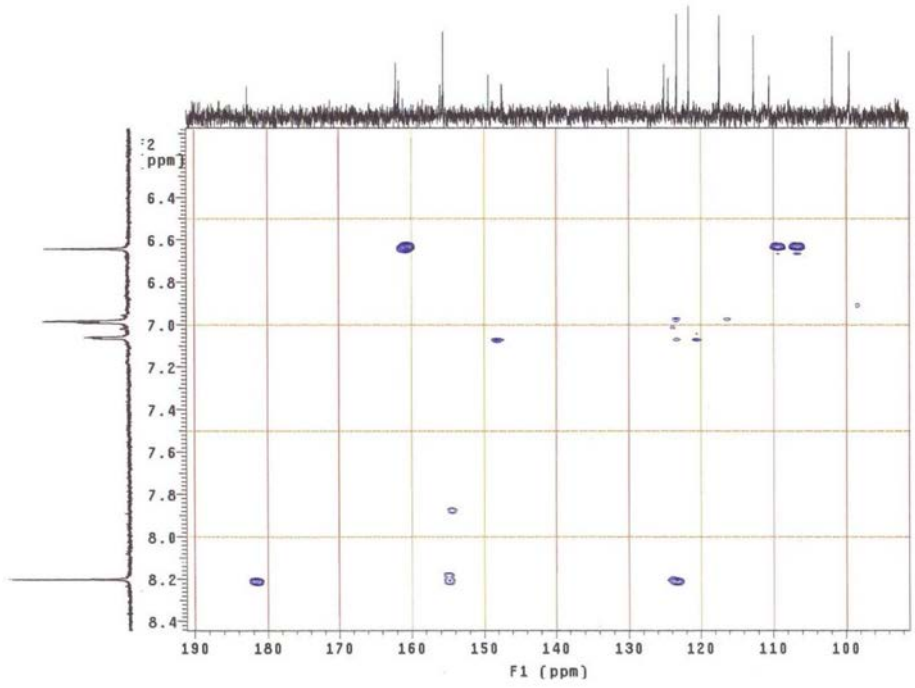

Figure S22. HMBC NMR spectrum of **3** (CD_3OD, 300 x 75 MHz) (expansion 1).

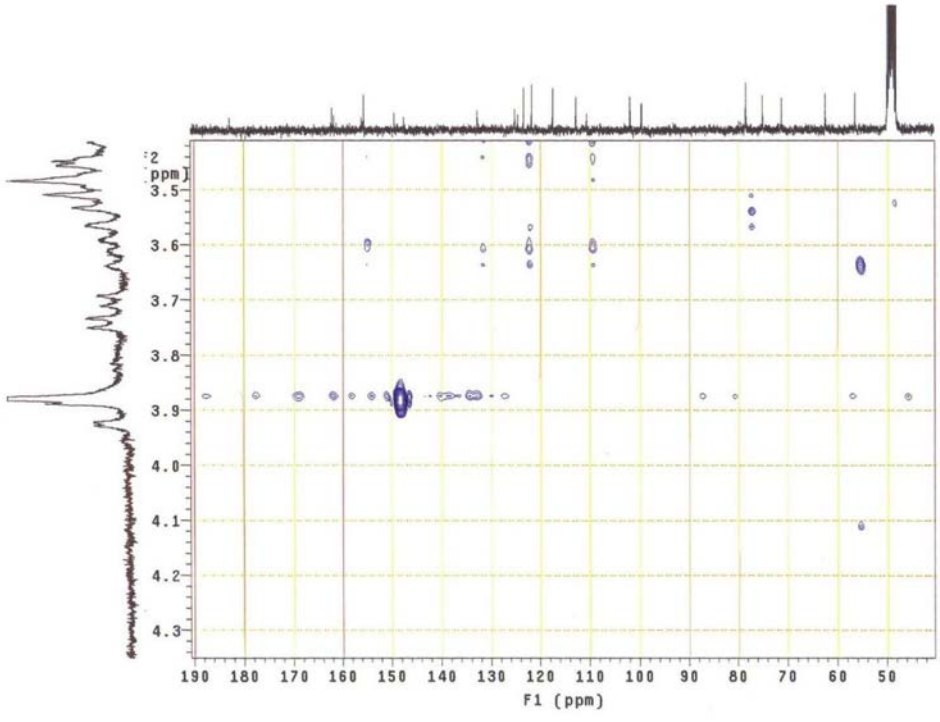

Figure S23. HMBC NMR spectrum of **3** (CD_3OD, 300 x 75 MHz) (expansion 2).

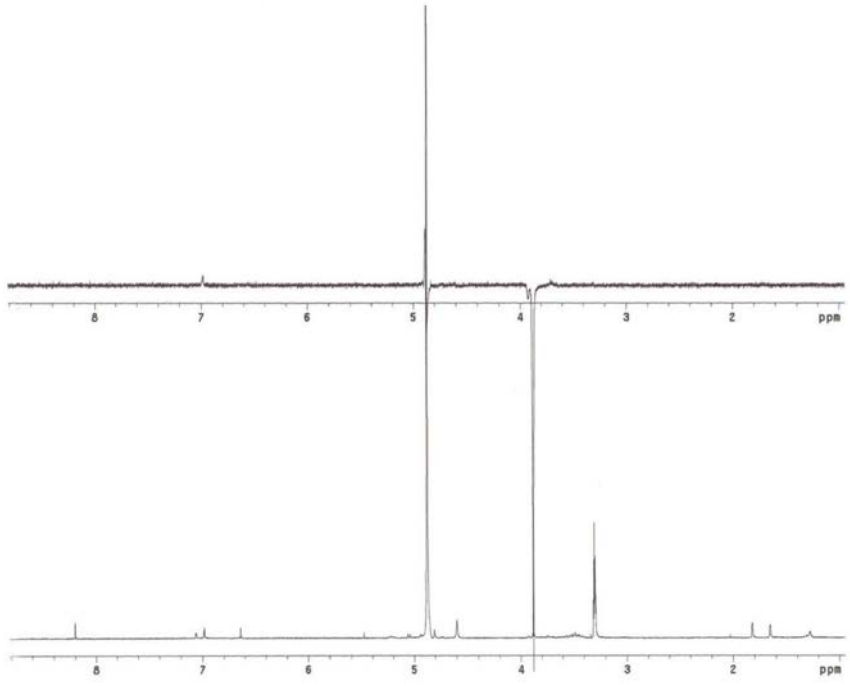

Figure S24. NOEdiff NMR spectrum of **3** (CD_3OD, 75 MHz).

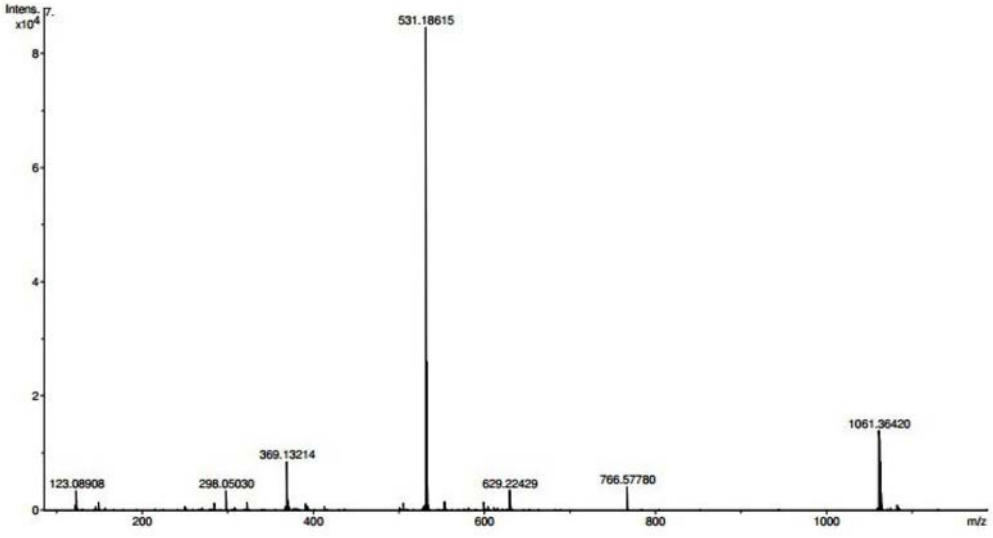

Figure S25. HRESITOF-MS spectrum of **3.**

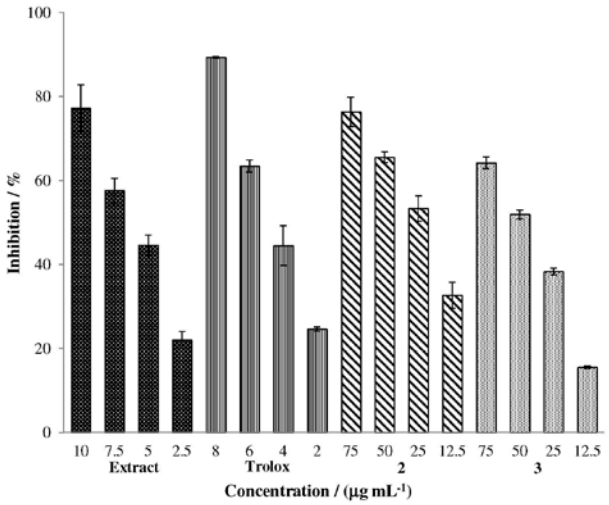

Figure S26. Percentage of inhibition of radical DPPH• caused by ethanolic extracts of the leaves of *V. guianensis*, isoflavones **2, 3** and positive control (Trolox).