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Article

Chromatographic profile of xanthones and flavonoids in the anti-dengue extracts of *Fridericia samydoides* (Cham.) L.G. Lohmann (Bignoniaceae)

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The flavonoids and xanthones present in the ethanol extracts of leaves and stems of *Fridericia samydoides* showed that anti-dengue activities *in vitro* were investigated qualitatively by liquid chromatography–ultraviolet-mass spectrometry in series. Nineteen flavones and fifteen xanthones were detected and characterized on the basis of their fragmentation pattern in the positive and negative ion mode tandem mass spectrometry spectra and ultraviolet bands. Acacetin, chrysin, vitexin, isovitexin, orientin, isoorientin, mangiferin, 2'-*O-trans*-caffeoylmangiferin, 2'-*O-trans*-coumaroylmangiferin and 2'-*O-trans*-cinnamoylmangiferin were identified by comparison with authentic samples. The other compounds detected were tentatively assigned by analysis of the spectral data and by comparison with literature reports. In addition, it performed the fractionation of the leaves extract leading to the isolation of mangiferin, isovitexin and isoorientin. All extracts and isolated compounds inhibited the Dengue virus replication cycle with EC_{50} less than 25.0 µg/mL for extracts and 272.5, 85.6 and 79.3 µg/mL for mangiferin, isovitexin and isoorientin, respectively.

Keywords: *Fridericia samydoides*; Antiviral activity; Flavonoids; *C*-glycosyxanthone; *Dengue type 2.*

INTRODUCTION

Plants afford an extensive chemical diversity and represent rich and renewable sources of natural products with promising biological activities. Antiviral of ethnomedicinal origin are of great interest and have been widely explored (Chattopadhyay, Naik, 2007). Dengue fever is a public health problem in tropical and subtropical areas of the world. In recent years, the incidence of dengue in the world has increased enormously. According to a recent estimate, 390 million dengue infections occur each year, of which 96 million are clinically manifested (Bhatt *et al.,* 2013; WHO, 2018).

The genus *Fridericia* belongs to the Bignonieae tribe (Bignoniaceae family), a large clade of Neotropical lianas that occur in South and Central America with rare representatives in North America (Gentry, 1992; Lohmann, 2006). In Brazil, species of this genus are present in the "*cerrado*" biome (savannahs) and are commonly found in the edges of riparian forests (Scudeller, Carvalho-Okano, 1998). Recently, research using molecular biology techniques has promoted changes in the classification of plant species. In this context, species of the genus *Arrabidaea* that have varied morphological characteristics have undergone changes in the botanical nomenclature of the species. Thus, the species *Arrabidaea samydoides*

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(Cham.) Sandwith is now a synonym name for *Fridericia samydoides* (Cham.) L.G. Lohmann) (Lohmann, 2006).

Some *Fridericia* species are reported as anti-inflammatory, astringent, anti-syphilitic, and are used in different South American countries for the treatment of skin diseases, leucorrhea, leukaemia, anemia and diarrhea (Brandão *et al.*, 2010a). We have previously reported the antiviral activity against Human herpesvirus 1 and Vaccinia virus Western Reserve of an *F. samydoides* ethanol extracts of leaves and stems (Brandão *et al*. 2010b).

Mass spectrometry currently plays a critical role in the discovery of natural products. It provides fast identification of known metabolites present in complex mixtures using small quantities of crude material and avoids time-consuming isolation procedures (Dinan, 2006). The association of chromatographic and spectroscopic techniques, such as LC-UV, LC-MS, LC-MS/MS, and GC-MS, has made possible the identification of new compounds present in complex mixtures as is the case of the vegetal extracts. All these factors have contributed to a rapid development of research in this area and also lowered the cost in the search for new bioactive compounds.

The aim of this work was to identify the components of ethanolic extracts from stems and leaves of *F. samydoides* using liquid chromatography coupled mass spectrometry and ultraviolet detector. Additionally, the anti-dengue activity of ethanol extracts from stems, leaves and isolated compounds was evaluated.

MATERIAL AND METHODS

General procedures for spectrometric data

The isolated compounds were submitted to ^{1}D and ^{2}D ^{1}H and ^{13}C-NMR spectra such as COSY, HSQC, HMBC and NOESY were obtained on a Bruker Avance DRX400 instrument. IR spectra were acquired on a Varian FT-IR Spectrometer using a Universal Attenuated Total Reflectance Accessory (UATR) in the range of 400 to 4,000 cm^{-1}. The melting points were determined on a Digital Melting apparatus (Microquímica). Determinations were performed at a heating velocity of 2 °C/min and were not corrected.

Collection, botanic identification and processing of plant materials

Fridericia samydoides (Cham.) Sandwith was collected in Santo Antônio do Monte, Minas Gerais, Brazil. The taxonomic determination of the plant was carried out by Dr. J.A. Lombardi, Department of Botany, Institute of Biosciences, UNESP, Rio Claro, Brazil. A voucher specimen was deposited at the BHCB/UFMG, Belo Horizonte, Minas Gerais, Brazil, under the number 23896. This work was registered in SisGen with accession number A083B26.

Extracts preparation of *F. samydoides*, chromatographic analysis and isolation procedures

The leaves and stems were dried in a ventilated oven at 40 °C for 72 h, ground (100 g of leaves and 75 g of stems) and extracted by percolation with 96% ethanol (5 x 250 mL) at room temperature. Ethanol was removed using a rotatory evaporator under reduced pressure at 50 °C, yielding 14.2 g of leaf extract and 7.3 g of stem extract.

LC-DAD-MS and LC-ESI-MS/MS analyses

LC-DAD-MS and LC-ESI-MS/MS analyses were performed using an UPLC Acquity (Waters) ion trap mass spectrometer in the following conditions: positive and negative ion mode; capillary voltage, 3500 V; capillary temperature, 320 °C; source voltage, 5 kV; vaporizer temperature, 320 °C; corona needle current, 5 mA; and sheath gas, nitrogen, 27 psi. Analyses were run in the full scan mode (100 – 2000 Da). The ESI-MS/MS analyses were additionally performed in an UPLC Acquity (Waters) with argon as the collision gas, and the collision energy was set at 30 eV. Chromatographic separation was done on ACQUITY UPLC BEH (1.7 μm, 50 × 2 mm i.d.) (Waters). The mobile phase consisted of water 0.1% formic acid (solvent A) and acetonitrile 0.1 % formic acid (solvent B). The elution protocol was 0 – 11 min, linear gradient from 5 % to 95 % B. The flow rate was 0.3 mL min^{-1}, and the sample injection volume was 4.0 μL. The UV spectra were registered from 190 to 450 nm. Mass spectrometry analysis was

performed a Waters ACQUITY® TQD equipped with on quadrupole instrument fitted with an electrospray source in the positive and negative ESI mode. Ion spray voltage: −4 kV; orifice voltage: −60 V.

Isolation of chemical components from leaves extracts

A portion leaves ethanol extract of *F. samydoides* (12.0 g) was transferred to a beaker and added to 300 mL of methanol. The mixture was allowed to stand to form a precipitate, which was separated by filtration on sintered glass funnel and washed thoroughly with methanol, yielding 2.1 g of a solid (ASF-PPTO-1) that was recrystallized from hot methanol to give 1015.0 mg of a solid referred to as ASF-1, then it was, subsequently, identified as being mangiferin.

The filtrate obtained from the initial extraction was dried and the residue (6.5 g) was subjected to filtration on silica gel column (30 × 3.8 cm i.d.) employing hexane, methylene chloride, ethyl acetate, ethyl acetate / methanol (2: 1), ethyl acetate / methanol (1: 2), methanol, methanol / water (1: 1) as eluents. An aliquot of fraction ethyl acetate / methanol (1: 2) (1.5 g) was fractionated on Sephadex LH 20 column to give 55 fractions. The fractions were pooled according to their TLC profile, yielding eight sub fractions. The sub fractions 5 (256.0 mg) and 6 (234.0 mg) were separately dissolved in methanol and subjected to a fractionation employing a preparative High Performance Liquid Chromatography (HPLC), then the ODS column Agilent Prep 410910-102 was employed, proceeding to the elution with linear gradient methanol/water 45 – 95 % for 50 min, flow 5 mL/min and UV detection at 220 nm. In the fractionation of subfraction 5, two called solid ASF- 2 (23.7 mg) were obtained; while the fractionation subfraction 6 obtained a solid called ASF- 3 (17.3 mg).

Spectrometric data for isolated compounds

Mangiferin (ASF-1): Yellow powder (MeOH); m.p. decomposes at 263.0-274; UV (MeOH) λ_{max} 239, 259, 316, 364 nm; IR ν_{max} 3373, 3183, 2925, 2889, 1647, 1621, 1589, 1563, 1523, 1493, 1465, 1401, 1349, 1293, 1253, 1195, 1087, 1033, 879, 821, 736 cm^{-1}; ^{1}H NMR (DMSO-d6, 400 MHz): **δ** 13.75 (s, 1H, 1-OH), 10.65 (s, 2H, 6,9-OH), 7.34 (s, 1H, H-8), 6.87 (s, 1H, H-5), 6.35 (s, 1H, H-4), 4.89 (s, 2H, 3',4'-OH), 4.57 (d, 10.0 Hz, 1H, H-1'), 4.46 (s, 1H, 6'-OH), 4.02 (t, 8.4 Hz, 1H, H-2'), 3.72 (d, 11.2 Hz, 1H, H-$6'_B$), 3.39 (m, 1H, H-$6'_A$), 3.17 (m, 1H, H-3'), 3.17 (m, 1H, H-4'), 3.17 (m, 1H, H-5'). ^{13}C NMR (DMSO-d6, 100 MHz): **δ** 179.3 (C, C-9), 164.0 (C, C-3), 161.9 (C, C-1), 156.4 (C, C-4a), 154.1 (C, C-6), 149.9 (C, C-10a), 143.6 (C, C-7), 111.5 (C, C-8a), 108.3 (C, C-8), 107.6 (C, C-2), 102.4 (C, C-5), 93.1 (C, C-4), 81.6 (C, C-5'), 79.2 (C, C-3'), 73.3 (C, C-1'), 70.7 (C, C-4'), 70.4 (C, C-2'), 61.5 (C, C-6'); HRESI-MS *m/z* 423.1023 $[M - H]^-$ (calcd for $C_{19}H_{19}O_{11}$, 423.0927).

Isovitexin (ASF-2). Yellow solid, m.p. 223–225 °C; (MeOH) λ_{max} 270, 336 nm; IR ν_{max} 3447, 3021, 2987, 1698, 1501, 1489, 1445, 1105, 1081, 837, 778 cm^{-1}; ^{1}H-NMR (DMSO-d6, 400 MHz): δ 7.94 (d, 8.5 Hz, 2H, H-3' and H-5'), 6.92 (d, 8.5 Hz, 2H, H-2' and H-6'), 6.76 (s, 1H, H-3), 6.50 (s, 1H, H-8), 4.58 (d, 8.9 Hz, 1H, H-1''), 4.02 (m, 1H, H-2''), 3.69 (m, 1H, H-6''b), 3.40 (m, 1H, H-6''a), 3.19 (m, 1H, H-3''), 3.17 (m, 1H, H-5''), 3.16 (m, 1H, H-4''). ^{13}C-NMR (DMSO-d6, 100 MHz): **δ** 182.4 (C=O, C-4), 163.7 (C, C-2 and C-7), 161.3 (C, C-4'), 160.9 (C, C-5), 156.5 (C, C-9), 128.9 (CH, C-6'), 128.5 (CH, C-2'), 121.7 (C, C-1'), 116.4 (CH, C-3' and C-5'), 109.8 (C, C-6), 103.8 (C, C-10), 103.2 (CH, C-3), 94.1 (CH, C-8), 82.0 (CH, C-5''), 79.4 (CH, C-3''), 73.5 (CH, C-1''), 71,0 (CH, C-2''), 70.7 (CH, C-4''), 61.9 (CH_2, C-6'').

Isoorientin (ASF-3). Yellow solid, m.p. 247–249 °C; UV (MeOH) λ_{max} 269, 349 nm; IR ν_{max} 3435, 3018, 2976, 1679, 1507, 1491, 1448, 1109, 1068, 811, 755 cm^{-1}; ^{1}H-NMR (DMSO-d6, 400 MHz): δ 13.56 (s, 1H, OH-5), 7.43 (d, 8.4 Hz, 1H, H-6'), 7.40 (s, 1H, H-2'), 6.91 (d, 8.4 Hz, 1H, H-5'), 6.68 (s, 1H, H-3), 4.49 (s, 1H, H-8), 4.57 (d, 8.8 Hz 1H, H-1''), 4.04 (m, 1H, H-2''), 3.68 (m, 1H, H-6''b), 3.37 (m, 1H, H-6''a), 3.20 (m, 1H, H-3''), 3.17 (m, 1H, H-5''), 3.15 (m, 1H, H-4''). ^{13}C-NMR (DMSO-d6, 100 MHz): **δ** 163.7 (C, C-2), 163.1 (C, C-7), 161.2 (C, C-5), 157.1(C, C-9), 150.5 (C, C-4'), 146.4 (C, C-3'), 122.1 (C, C-1'), 119.6 (CH, C-6'), 115.9 (CH, C-5'), 113.8 (CH, C-2''), 103.9 (C, C-10), 102.7 (CH, C-3), 93.4 (C, C-8), 81.5 (CH, C-5''), 78.8 (CH, C-3''), 72.9 (CH, C-1''), 70.5 (CH, C-2''), 70.1 (CH, C-4''), 61.4 (CH_2, C-6'').

Cell culture and virus

Kidney cell LLC-MK2 (ATCC® CCL-7™) and normal human lung fibroblast cell MRC-5 (ATCC® CCL-117™) were cultured in Dulbecco's modified Eagle's medium (DMEM, Cultilab, Campinas, SP, Brazil) at 37 °C, in 5% CO_2 atmosphere, supplemented with 5% fetal bovine serum, 50 µg/mL gentamicin, 100 U/mL penicillin and 5 µg/mL amphotericin B (Brandão *et al.*, 2017).

DENV-2, was kindly donated by Dr. L. Figueiredo (USP, Ribeirão Preto, Brazil), Dr. I. Kerr (London Research Institute, London, UK). The virus was titrated by $TCID_{50}$ in LLC-MK2 cells and the titers was 1.0×10^4 $TCID_{50}$/mL (Rodriguez *et al.*, 1990).

Cytotoxicity assay

LLC-MK2 and MRC-5 cells were exposed to different concentrations (800 to 0.125 µg/mL) of extracts/fractions/compounds for 72 h (Brandão *et al.*, 2017). After incubation, cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Merck) assay at a concentration of 2 mg/mL in phosphate buffered saline (PBS) (Brandão *et al.*, 2017; Twentyman, Luscombe, 1987).

Antiviral assays

The antiviral activity (EC_{50}) was evaluated by the MTT assay (Betancur-Galvis *et al.*, 1999) α-2a interferon (Bergamo Brazil, São Paulo, SP, Brazil) was used as positive control (Brandão *et al.*, 2017). Experiments were carried out with eight different concentrations (500 to 0.125 µg/mL) within the inhibitory range of the samples. The 50% inhibitor concentration of the viral effect (EC_{50}) for each extract, fractions, and constituents were calculated from concentration-effect-curves after no linear regression analysis (Brandão *et al.*, 2017). The selective index (SI) is defined as CC_{50} over EC_{50}. Statistical calculations were carried out with the GraphPad prism 5.0 software package (Statistica). Results are expressed as the mean ± S.E.M. of 3 independent experiments. The student's t-test was used for statistical analyses; P values > 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Chemical analyses

Screening, identification, and further confirmation of many components in *F. samydoides* ethanol extracts were performed by LC–DAD-MS, and used to obtain molecular mass ions as well as characteristic fragment ions. Twelve isolated compounds (mangiferin, 2'-*O-trans*-caffeoylmangiferin, 2'-*O-trans*-coumaroylmangiferin, 2'-*O-trans*-cinnamoylmangiferin, acacetin, chrysin, vitexin, isovitexin, orientin, isorientin, vitexin-2"-*O*-glycoside and rutin) were used as standards for optimization in the separation of phenolic compounds using UPLC, and the ionization and fragmentation using ESI MS^2. The full scan mass spectrum (200-700 Da) obtained from *F. samydoides* ethanol extracts by LC–DAD-MS in negative and positive ion modes are presented in Figure 1. Sensitivity was greater when chromatograms were acquired in negative-ion mode than in positive-ion mode, in which, few peaks were observed. Tentative identification of plant components was performed by detailed fragmentation studies and by comparison with published data from the literature.

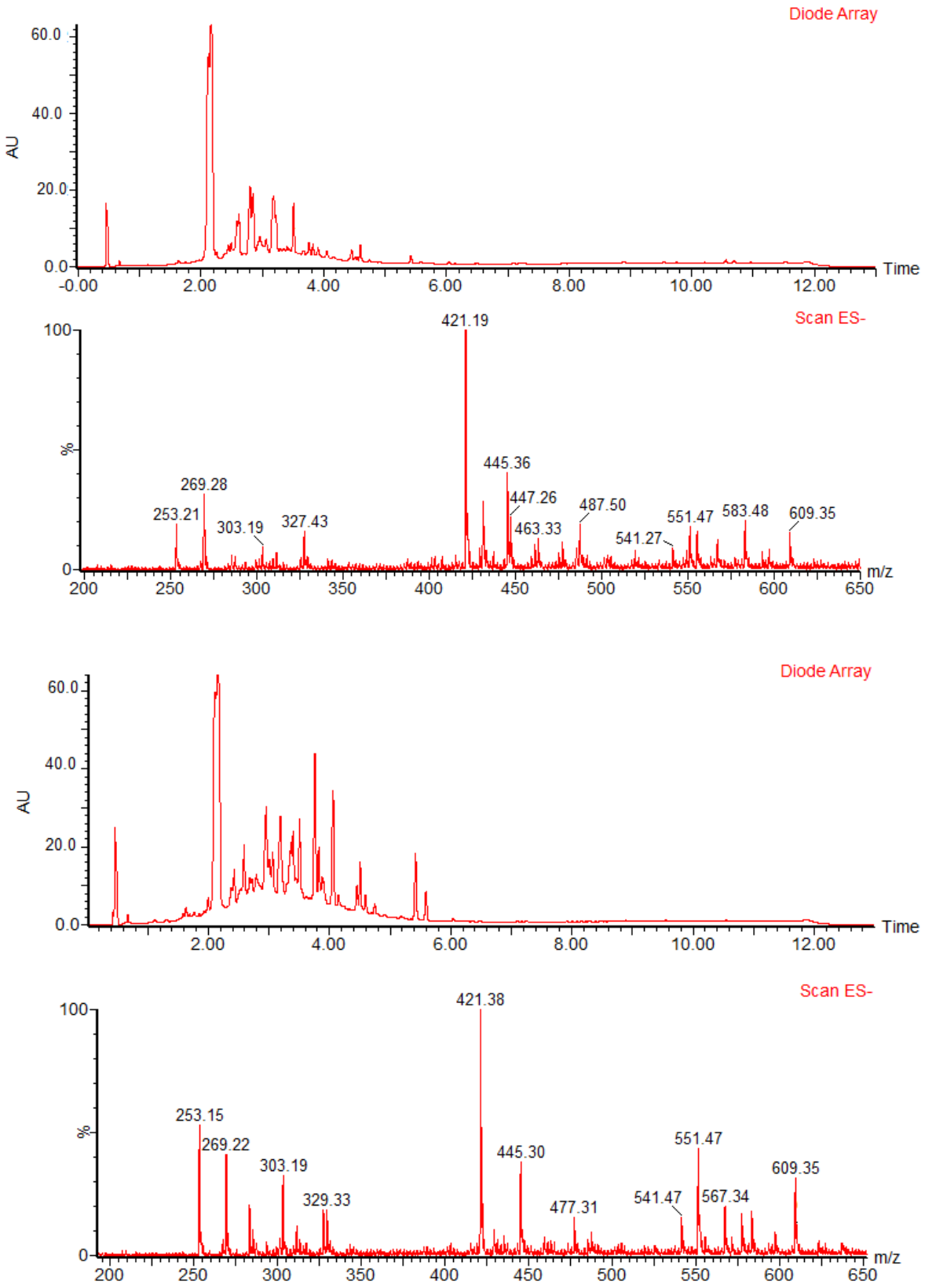
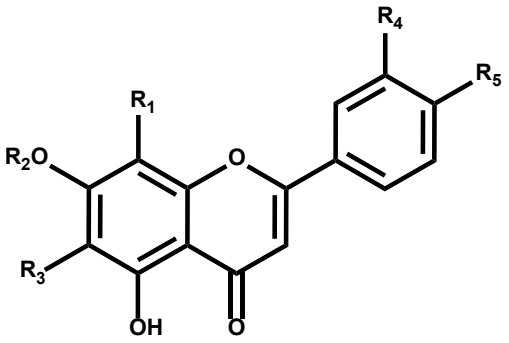


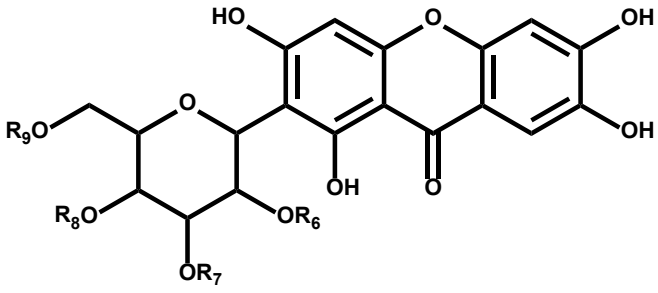
FIGURE 1 – UPLC fingerprint (A and C) and Full scan mass spectrum (B and D) of the ethanolic extracts from *Fridericia samydoides* leaves (A and B) and stems (C and D). Chromatographic conditions: see Experimental Section.

The fingerprinting obtained for ethanolic extract of stems and leaves of *F. samydoides* by UPLC-DAD showed the presence of a large amount of flavonoids and xanthones. The identified flavonoids can be divided into subgroups: *C*-glycosylflavones, *O*-glycosylflavones, *O* and *C*-glucosylflavones and non-glycosylated flavones (aglycone). Analysis of first-order MS spectra recorded for each peak together with MS^2 experiments in positive and negative ESI mode, UV comparison, and retention time (RT) led to the following structure assignments (Table I). The structures of the flavonoids identified in the ethanolic extract of *F. samydoides* leaves are shown in Figure 2.



Vitexin: R_1=Glyc, R_2=R_3=R_4=H, R_5=OH
Isovitexin: R_1=R_2=R_4=H, R_3=Glyc, R_5=OH
Orientin: R_1=Glyc, R_2=R_3=H, R_4=R_5=OH
Isoorientin: R_1=R_2=H, R_3=Glyc, R_4=R_5=OH
Isoviolanthin: R_1=Glyc, R_2=H; R_3=Ram, R_4=H, R_5=OH
Violanthin: R_1=Ram, R_2=H; R_3=Glyc, R_4=H, R_5=OH
Orientin-2"-*O*-glycosides: R_1= Glyc, R_2=R_3=H, R_4=R_5=OH
Scutellarein 7-*O*-glucuronide: R_1=H, R_2=Gluc, R_3=OH, R_4=OH, R_5=H
6-methoxyluteolin -7-*O*-glucuronide: R_1=H, R_2=Gluc, R_3=OCH_3, R_4=OH, R_5=OH
Chrysin-7-*O*-glucuronide: R_1=R_3=R_4=R_5=H, R_2=Gluc

Acacetin-7-*O*-glucuronide: R_1=R_5=H, R_2=Gluc, R_3=OH, R_4=OCH_3
Hispidulin -7-*O*- glucuronide: R_1=R_5=H, R_2=Gluc, R_3= OCH_3, R_4=OH
Apigenin - 7-*O*- glicuronide: R_1=R_3=R_5=H, R_2=Gluc, R_4=OH
Apigenin- 4-*O*- glicuronide: R_1=R_2=R_3= R_5=H, R_4=*O*-Gluc
6-hydroxyluteolin-7-*O*-glucoside: R_1=R_5=H, R_2=Glyc, R_3=R_4=OH
8-hydroxyluteolin-7-*O*-glucoside: R_1=OH, R_2=Glyc, R_3=R_5=H, R_4=OH
Chrysin: R_1=R_2=R_3=R_4=R_5=H
6-hydroxy-chrysin: R_1=R_2=R_4=R_5=H, R_3=OH
Acacetin: R_1=R_2=R_3=R_4=H, R_5=OCH_3
6- methoxyluteolin: R_1=R_2=H, R_3=OCH_3, R_4=R_5=OH
8- methoxyluteolin: R_1=OCH_3, R_2=R_3=H, R_4=R_5=OH



Mangiferin: R_1=R_2=R_3=R_4=H
2"-*O*-*trans*-benzoylmangiferin: R_1=Benzoyl, R_2=R_3=R_4=H
2"-*O*-*trans*-cinnamoylmangiferina: R_1=Cinnamoyl, R_2=R_3=R_4=H
2"-*O*-*trans*-coumaroylmangiferin: R_1=Coumaroyl, R_2=R_3=R_4= H
2"-*O*-*trans*-caffeoylmangiferin: R_1=Caffeoyl, R_2=R_3=R_4=H

FIGURE 2 – Xathones and flavonoids identified in ethanolic extract from the *Fridericia samydoides.*

The fragmentation of flavone *C*-glycosides did not observe the presence of the aglycone ion due to resistance of the C-C connection between the sugar molecule and the aglycone. This behavior was observed in first-order

MS of compounds 1, 2, 3, 4, 5, and 6, thus suggesting that these compounds are flavones *C*-glycosides. The MS^2 spectra, obtained by focusing on each $[M - H]^-$ ion of compounds 1, 2, 3, 4, 5, and 6, exhibited the same pattern of fragmentation ($[(M - H) – 18\ Da]^-$, $[(M - H) – 90\ Da]^-$, $[(M - H) – 120\ Da]^-$, typical of *C*-glycosylflavones. Compound 1 and 2 with retention time (R_t) 2.58 and 2.80 min are isomers. The MS spectrum in negative mode focused on *m/z* 431.32 and 431.45 ($[M - H]^-$), respectively. The MS^2 spectrum in negative mode observed fragments of *m/z* 311.29 and 311.10 related to the loss of 120 Da. Additionally, it observed fragments of *m/z* 341.48 and 341.10 related to the loss of 90 Da. Data analysis and comparison with reference samples permitted the identification of compounds 1 and 2 as the vitexin and isovitexin, respectively. Using the same strategy employed in the identification of compounds 1 and 2, it was possible to identify the isomers orientin and isoorientin, violanthin and isoviolanthin. Compounds 3, 4, 5 and 6, respectively. Experimental data is shown in Table I.

TABLE I - Flavonoids identified in ethanolic extract of leaves from the *Fridericia samydoides*

	Compounds	RT (min)	UV (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	Fragments (*m/z*)
	***C*-glycosylflavones**					
1	Vitexin	2.58	268, 332	433.27	431.32	311.29, 341.48
2	Isovitexin	2.80	269, 327	433.34	431.45	311.10, 341.10
3	Orietin	2.45	258, 323	449.41	447.31	327.16, 357.69
4	Isoorientin	2.49	259, 324	449.48	447.25	327.29, 356.79
5	Violanthin	2.40	272, 315	579.36	577.34	457.20, 487.33
6	Isoviolanthin	2.42	271, 316	579.40	577.21	457.06, 486.69
7	Orientin-2”-*O*-glycoside	2.57	268, 327	611.50	609.46	447.32, 357.43, 327.40
	***O*-glycosylflavones**					
8	Scutellarein 7-*O*-glucuronide	2.84	280, 326	463.46	461.23	*463.20, 287.04
9	6-Methoxyluteolin 7-*O*-glycoside	2.72	270, 324	479.47	477.49	315.22, 301.07, 161.11
10	Chrysin-7-*O*-glucoronide	3.73	270, 324(sh)	431.44	429.22	253.25, 175.13
11	Acacetin 7-*O*-glucuronide	3.62	262, 323	461.30	459.21	459.21, 283.08, 268.16
12	Hispidulin 7-*O*-glglucuglucuronide	2.72	273, 323	477.31	475.45	*301.22, 285.83
13	Apigenin 7-*O*-glucuronide	3.18	264, 323	447.45	444.86	269.07
14	Apigenin 4’-*O*-glucuronide	4.55	280, 320	447.32	445.24	268.94
15	6(8)-hydroxyluteolin 7-*O*-glycoside	2.58	268, 331	465.28	463.13	*302.92
	Aglycones					
16	Chrysin	5.34	267, 319(sh)	255.24	253,21	211.05, 183.34, 153.06,103.17

TABLE I - Flavonoids identified in ethanolic extract of leaves from the *Fridericia samydoides*

	Compounds	RT (min)	UV (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	Fragments (*m/z*)
17	6-hydroxy-chrysin	4.38	285	271.24	269.21	253.04, 225.45, 165.08, 121.88, 106.83
18	Acacetin	5.52	270, 317	285.29.	283.13	267.96, 238.97, 222.77
19	6(8)- methoxyluteolin	4.50	272, 327	301.45	299.40	286.11, 167.09

*Fragmentation analyzes in positive mode

Compound 7 show the MS spectrum in negative mode focused on *m/z* 609.46 ($[M - H]^-$) was not particularly useful for structural identification; base peak *m/z* 447.32 was observed regarding the fragment loss of 162 Da, which can be attributed to a break hexoses unit. This type of break occurs only when the sugar moiety is linked O-C. Additionally, secondary fragments were observed with *m/z* 327.40 and 357.43 in the loss of 90 and 120 mass units characteristic of *C*-glucosylflavones. A comparative analysis on the fragmentation of compound 7 with vitexin-*2 "-O*-glycoside (*m/z* 593.34 $[M - H]^-$) observed similarity in the type of breakage, and the only difference is less 16 Da related to the residue fragment of C-glycosylflavone 431.28 for vitexin-*2 "-O*-glycoside, whereas, in compound 7 a fragment of 447.32 was observed. In view of these results and with the literature comparison data, it has been suggested that compound 7 could be the orientin-*2"-O*-glycoside/isoorientin-*2"-O*-glycoside.

The presence of *C*-glucosylflavones in extracts of Bignoniaceae species was previously reported to the *Arrabidaea chica, Macfadyena unguis-cati* and *Martinella obovata* (Aboutabl *et al.,* 2008; Arevalo *et al*., 2011; Barbosa *et al*., 2008; Duarte *et al*., 2000). The compounds vitexin, isovitexin and orientin were isolated from leaves extract of *M. obovata*, a species used in popular medicine of Honduras for the treatment of eye infections (Arevalo *et al.,* 2011). The isoorientin, isoviolantin/violantin and orientin-*2"-O*-glycoside/ isoorientin-*2"-O*-glycoside is the first report of these compounds in Bignoniaceae species.

Flavonoid *O*-glycosides are easily fragmented releasing the corresponding aglycone. Compounds 8, 9, 10, 11, 12, 13, 14 and 15 showed UV absorptions typical of flavone derivatives. Positive and negative ion MS^2 analysis allows for the identification of both the aglycones and the saccharide substituents. In Bignoniaceae species, the carbohydrate components are usually either monosaccharides (glucose, for instance) or disaccharides (rutinose or neohesperidose), and they are generally attached to the aglycone in positions 7 and 4' (Mabry, Markham, Thomas, 1970).

Compound 8 show the MS spectrum in positive mode focused on *m/z* 463.46 ($[M + H]^+$). The UV spectrum showed two λ_{max} at 280.1 nm and 326.1 nm, typical of flavones. The λ_{max} at 280.1 nm for band II chromophore is suggestive of increased oxygenation in the ring A. The positive mode MS^2 focused on *m/z* 463.20 showed the loss of a 176 Da fragment, which can be attributed to a break in glucuronic acid residue. Additionally, a base peak of *m/z* 287.04 $[(M+H) – 176]^+$ was observed regarding aglycone. Data analysis permitted the identification of compound 8 as the scutellarein 7-*O*-glucuronide.

Using similar analyzes of compound 8, it was possible to identify compounds 9, 10, 11, 12, 13, 14 and 15 as being 6-methoxyluteolin 7-*O*-glucoside, chrysin-7-*O*-glucuronide, acacetin 7-*O*-glucuronide, hispidulin 7-*O*-glucuronide, apigenin 7-*O*-glucuronide, apigenin 4'-*O*-glucuronide and 6(8)-hydroxyluteolin 7-*O*-glucoside, respectively.

The presence of the flavonoid *O*-glycosides in Bignoniaceae species is widely reported in the literature

(Blatt, Salatino, Salatino, 1996; Dinda, Debnath, Harigaya, 2007). In the genus *Arrabidaea,* the presence of this substance class only in extracts of *A. brachypoda* was reported (Blatt, Santos, Salatino, 1998). The apigenin-7-*O*-glucuronide was isolated from the extract of *Jacaranda ovalifolia* and *Millingtonia hortensis* (Gouda *et al*., 2003; Hase *et al*., 1995). The apigenin-7-*O*-glucoside is present in the extracts of *Mayodendron igneum*, *Macfadyena unguis-cati* and *Mansoa difficilis* (Aboutabl *et al*., 2007; Hashem, El-sawi, Sleem, 2007; Guilhon *et al*., 2012). Chrysin-7-*O*-glucuronide was isolated from *Oroxylum indicum* seeds, while acacetin 7-*O*-glucuronide was isolated from *Tecoma capensis* leaves (Marzouk, 2002; Wen, Zhang, Yin, 2011). The 6-methoxyluteolin 7-*O*-glucoside, hispidulin 7-*O*-glucuronide and apigenin 4'-*O*-glucuronide and 6(8)-hydroxyluteolin 7-*O*-glucoside identified in the *F. samydoides* extract first reported these compounds in Bignoniaceae species.

The aglycones with various hydroxyl substitutions, and methoxyl substitutions were detected in *F. samydoides* extracts. The ions produced from a retro-Diels-Alder (RDA) fragmentation reaction of the cleavage of the C-ring with UV data were used in identification of compounds. Other common secondary ion fragments such as loss of CO (28 Da) and CO_2 (44 Da) C-ring also contributed to characterization (Panda, 2010). Additionally, the compounds containing methoxyl groups gave an ion loss of CH_3 (15 Da), which was consistent with the literature (Wen, Zhang, Yin, 2011). In this way, the compounds 16, 17, 18 and 19 were identified as chrysin, 6-hydroxy-chrysin, acacetin and 6(8)-methoxyluteolin, respectively.

Chrysin is a flavone present in the extract of some species of Bignoniaceae including *F. samydoides*; and its derivative 6-hydroxy-chrysin also known as baicalein is extensively described as a constituent of *Oroxylum indicum* extracts (Pauletti, Bolzani, Young, 2003; Wen, Zhang, Yin, 2011). The methoxyapigenin (acacetin) flavone is a widespread occurrence in the plant kingdom. In Bignoniaceae, this flavonoid was isolated from the species *Macfadyena unguis-cati* and *O. indicum* (Aboutabl *et al*., 2007; Wen, Zhang, Yin, 2011). The 6-methoxyluteolin was isolated from *O. indicum* (Dinda *et al*.; 2007). Thus, all flavones aglycones identified in the leaves of ethanol extract of *F. samydoides* are widely spread in Bignoniaceae species extracts.

For identification and characterization of xanthones derivatives, the following points were considered UV spectrum, molecular ion peaks in positive and negative ion ESI mode of MS. The UV spectrum of glycosylated xanthones have some characteristics, and often differ from those shown by flavonoids, where the presence of three or more absorption bands often decrease intensity. In addition, four isolated xanthones (mangiferin, 2'-*O-trans*-caffeoylmangiferin, 2'-*O-trans*-coumaroylmangiferin, and 2'-*O-trans*-cinnamoylmangiferin) were used as references. LCMS data of the xanthones present in the *F. samydoides* leaves ethanolic extracts are summarized in Table II and their structures are shown in Figure 2.

TABLE II - Xanthones identified in ethanolic extract of leaves from the *Fridericia samydoides* negative ion mode

	Compounds	RT (min)	UV (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	Fragments (*m/z*)
20	Mangiferin	2.17	239, 257, 318, 364	423.34	421.38	*301.21, 330.86
21	Isomagiferin	2.01	238, 257, 318, 362	423.41	421.35	*300.96, 330.98
22	Mangiferin benzoyl derivative	2.92	258, 280, 319	543.30	541.46	*421.74, 402.95, 282.87
23	Mangiferin benzoyl derivative	3.02	259, 280, 320	543.43	541.14	*419.59, 403.14, 282.93
24	2''-*O-trans*-cinnamoylmangiferin	3.82	259, 280, 316	553.36	551.35	404.87, 285.24, 131.04

TABLE II - Xanthones identified in ethanolic extract of leaves from the *Fridericia samydoides* negative ion mode

	Compounds	RT (min)	UV (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	Fragments (*m/z*)
25	Mangiferin cinnamic derivative	3.85	260, 280, 320	553.49	551.85	404.68, 284.85, 130.99
26	Mangiferin cinnamic derivative	4.00	259, 280, 320	553.56	551.60	404.87, 284.98, 131.17
27	2''-*O-trans*-coumaroylmangiferin	3.76	260, 280, 319	569.33	567.34	303.13, 273.17, 147.80
28	Mangiferin coumaroyl derivative	3.62	260, 280, 319	569.36	567.27	303.06, 273.16, 147.18
29	Mangiferin coumaroyl derivative	3.71	262, 320	569.47	567.40	302.87, 273.04, 147.12
30	2'-O-trans-coumaroylmangiferin	3.83	259, 280, 316	569.52	567.39	302.93, 285.26, 147.06
31	2''-*O-trans*-caffeoylmangiferin	3.51	259, 280, 320	585.22	583.89	405.26, 303.11, 284.92, 163.08
32	Mangiferin caffeoyl derivative	3.26	258, 280, 319	585.79	583.20	405.20, 303.12, 285,93 163.27
33	Mangiferin caffeoyl derivative	3.45	259, 280, 318	585.16	583.13	404.82, 302.92, 284.99, 162.90
34	2'-O-trans-caffeoylmangiferin	3.02	257, 280, 318	585.51	583.58	405.13, 303.17, 285.36, 163.02

*Fragmentation analyzes in negative mode

Compounds 20 and 21 with R_t 2.17 and 2.01 min are isomers. The MS spectrum in negative mode showed deprotonated molecule with *m/z* 421.38 and 421.35 ($[M - H]^-$), respectively. In the MS^2 spectrum analysis fragments of *m/z* 301.21 and 300.96 related to the loss of 120 Da and fragments of *m/z* 330.86 and 330.98 related to loss 90 Da characteristic of *C*-glycosyl residues were observed. Data analysis and comparison with reference samples permitted the identification of compounds 20 and 21 as the mangiferin and isomagiferin, respectively.

The compounds 22 and 23 with retention times 2.92 and 3.02 min. showed the deprotonated molecule $[M - H]^-$ *m/z* 541.46 and 541.14 ($[M - H]^-$), respectively. In the MS^2 experiments, in negative mode (ESI^-) fragments of *m/z* 421 were observed for both compounds, attributed to the loss of the benzyl group (120 Da), followed by a dehydration fragment ion *m/z* 403. Furthermore, a fragment of *m/z* 283 related drop in residue *C*-glycosyl-xanthone (120 Da) was observed. These data suggest that compounds 22 and 23 are benzoyl derivatives of mangiferin.

The compounds 24, 25 and 26 showed the deprotonated molecule *m/z* 551.35, 551.85 and 551.60 ($[M - H]^-$). MS^2 experiments in negative mode (ESI) observed ion fragments similar to the three compounds. The ion fragment *m/z* 404 attributed to the loss of a cinnamoyl radical followed by dehydration [(551-138)-18] and *m/z* 284 related drop in residue *C*-glycosyl-xanthone (120 Da). The compound 24 was identified by comparison with a reference substance and identified as 2'-*O-trans*-cinnamoylmangiferin. Since compounds 25 and 26 showed similar spectral data to compound 24, hence suggesting that compounds 25 and 26 are also cinnamic derivatives of mangiferin.

The compounds 27, 28, 29 and 30 are also isomers with retention times 3.76, 3.62, 3.71 and 3.83 min. The MS spectrum in positive mode showed the protonated molecules $[M+H]^+$ *m/z* 569.33, 569.36, 569.47 and 569.52, respectively. All compounds show UV spectra similar to the *C*-glycosyl-xanthone. The MS^2 experiments in negative mode (ESI) observed base peak *m/z* 147 Da for the four isomers, suggesting the presence of coumaric acid residue in the structures of the compounds. Other similar fragments were observed in the analysis of the four isomers (Table II). The compound 30 was identified by comparison with a reference substance and identified as 2'-*O*-*trans*-coumaroylmangiferin. Since compounds 27, 28 and 29 showed similar spectral data to compound 30, thus it suggests that the compounds 27, 28 and 29 are also coumaroyl derivatives of mangiferin.

Compounds 31, 32, 33 and 34 also showed a UV spectrum similar to *C*-glycosyl-xanthone. In the positive mode (ESI^+) analysis, protonated molecules of *m/z* 585.22, 585.79, 585.16 and 585.51 were observed, while in the negative mode (ESI^-), deprotonated molecules of *m/z* 583.89, 583.20, 583.13 and 583.58. In the fragmentation experiments (ESI^+), the loss of fragments with 163.0 Da related to the loss of caffeic acid residue was observed, in addition another fragment of *m/z* 405 common to the four isomers was observed, this fragment may be related to the loss of the caffeoyl radical plus a water molecule [(M+H) -162] -18 Da. Furthermore, fragments of *m/z* 303 and 285 related to the drop in residue C-glycosyl xanthone loss of 120 Da [(M+H)-120] and (405-120 Da) were observed. The compound 34 was identified by comparison with a reference substance and identified as 2'-*O*-*trans*- caffeoylmangiferin. The compounds 31, 32 and 33 showed similar spectral data to compound 34, which allows one to suggest that the compounds 31, 32 and 33 are also caffeoyl derivatives of mangiferin.

The first occurrence report of xanthones in Bignoniaceae family was published by Pauletti *et al.* (2003). They isolated mangiferin and five stems extract derived from this species (muraxanthone, 2'-*O*-*trans*-benzoylmangiferin, 2'-*O*-*trans*-caffeoylmangiferin, 2'-*O*-*trans*-coumaroylmangiferin and 2'-*O*-*trans*-cinnamoylmangiferin). Subsequently, Martin *et al.* (2008) reported the presence of this natural product class of *Arrabidaea patellifera* leaves extract from which they isolated mangiferin, isomangiferina and six new derivatives (3′-*O*-*p*-hydroxybenzoylmangiferin, 3′-*O*-*trans*-coumaroylmangiferin, 6′-*O*-*trans*-coumaroylmangiferin, 3′-*O*-*trans*-cinnamoylmangiferin, 3′-*O*-trans-caffeoylmangiferin, and 3′-*O*-benzoylmangiferin). Brandão *et al.* (2010b) also isolated mangiferin of stems and leaves extracts of *F. samydoides*. The present study identified the presence of fifteen xanthones in the extract from the leaves of this species.

Isolated, identification and anti-dengue activity of extract and substances from *F. samydoides*

The phytochemical investigation of the ethanolic extract of *F. samydoides* leaves led to the isolation and structural elucidation of three compounds (Figure 3). The mangiferin (ASF-1) xanthone commonly isolated from *Mangifera indica* leaves was obtained in a large amount extraction with methanol cold. This compound was previously isolated from this species (Brandão *et al*., 2010b; Pauletti *et al*., 2003). The identification was performed by comparison with an authentic sample (Brandão *et al*., 2010b). Additionally, two other compounds were isolated. In the UV tests, two absorption maximums characteristic of flavonoids were observed. The ^1H and ^{13}C NMR data, in addition to the two-dimensional analyzes (HSQC and HMBC), as well as a comparison with literature data, allowed the identification of ASF-2 and ASF-3, such as isovitexin and isoorientin, respectively (Cheng *et al*., 2000; Kato, Morita, 1990).

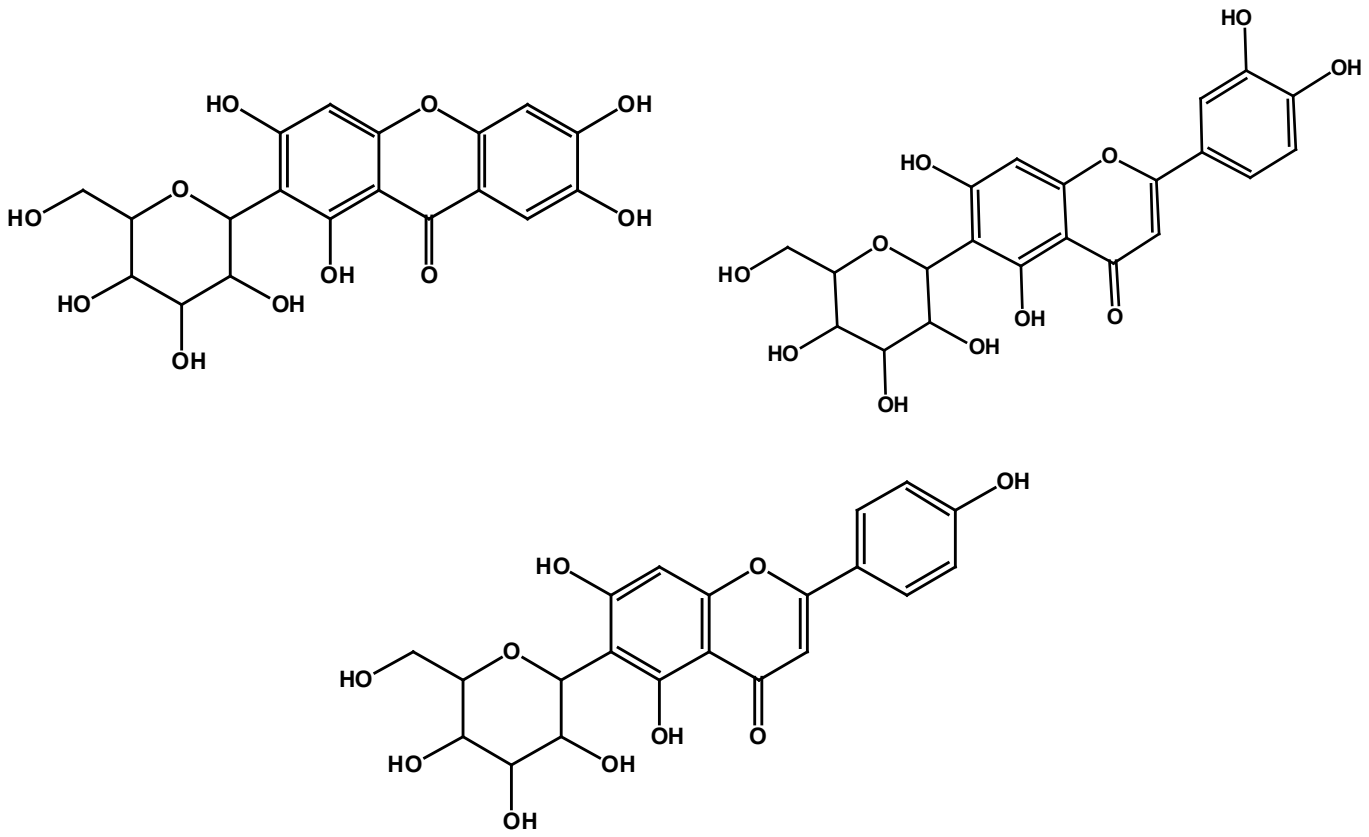


FIGURE 3 – Isolated compounds in ethanolic extract of leaves from the *Fridericia samydoides.*

As a result of the great activities observed for the ethanol extracts of *F. samydoides* against DENV-2, the isolated compounds were all evaluated for their anti-dengue activities (Table III). As shown in Table III, mangiferin (ASF-1), isovitexin (ASF-2), and isoorientin (ASF-3) were all active *in vitro* against the DENV-2. However, they were less active than the ethanol extracts. Reports of flavonoid as anti-viral agents are numerous, and even the mode of action has been proposed (Chattopadhyay, Naik, 2007). Despite the several reports of antiviral activity of flavonoids, this study reported this activity for isotexin and isoorientin for the first time. Recently, the aglycones present in these two compounds (apigenin and luteolin) presented anti-chikungunya activity, which contributes to the findings of the present study (Murali *et al*., 2015). Brandão *et al.* (2017) isolated three mangiferin derivatives from the ethanol extract of *Fridericia formosa*. These xanthones showed high antiviral activity with EC_{50} ranging from 2.4 to 4.5 µg/mL against Dengue virus 2. Furthermore, these xanthones were active against HSV-1 and Vaccinia virus WR. Once again, suggesting that the constituents present in extracts of *F. samydoides* may become promising antiviral drugs.

TABLE III - Cytotoxicity (CC_{50}, LLC-MK2 and MRC-5 cells), *in vitro* antiviral activity (EC_{50}), selectivity index (SI) for ethanol extracts from *Fridericia samydoides* leaves (EEASL), stems (EEASS) and compounds 1 – 3

Extracts/Compounds	LLC-MK2 CC_{50} µg/mL	[a]DENV-2 EC_{50} µg/mL	SI	MRC-5 CC_{50} µg/mL
EEASL	93.6 ± 4.2	< 25.0	> 3.7	> 200
EEASS	> 500	< 25.0	> 3.8	>200
Mangiferin	> 500	272.5 ± 6.7	> 1.8	>200
Isovitexin	> 500	85.6 ± 4.8	5.8	>200
Isoorietin	> 500	79.3 ± 3.2	6.3	>200
Interferon a	-	[bc]2.5 x 10^3	-	-

[a]Viral titer $TCID_{50}$/mL 1.0 × 10^4 in 72 h; [b]80 to 100% inhibition of cytopathic effect; [c]concentration in UI/mL

CONCLUSIONS

The results of this study show that flavonoids and xanthones are the major phenolic constituents of *F. samydoides* extracts. The analysis by UPLC-UV-MS provided important information on the structure of these constituents shown to be a useful tool for the study of plant extracts.

The anti-dengue activity of the isolated compounds was moderate. The activity of the ethanol extracts was higher when compared to the isolated substances. This fact can be explained by the presence of mangiferin derivatives in ethanolic extracts whose anti-dengue activity was described recently (Brandão *et al*., 2017). This suggests that *F. samydoides* is a potential source of anti-dengue compounds.

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