Flavonol glycosides from the leaves of **Boldoa purpurascens** and their anti-inflammatory properties

Flavonol glycosides from the leaves of *Boldoa purpurascens* and their anti-inflammatory properties

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Abstract

Boldoa purpurascens is used in Latin America and the Caribbean as a potent diurectic. Phytochemical analysis has shown the presence of flavonoids and other active compounds. In the present work, three flavonol glycosides were isolated from the leaves of the plant. Their structures have been determined by mass spectrometry and by 1D and 2D NMR analysis as 6-methoxykaempferol-3-*O*-[α-L-rhamnopyranosyl-(1”’→2”)]-β-D-xylopyranoside (**1**); 3,4',5-trihydroxy-6,7-methylene-dioxyflavone-3-*O*-[α-L-rhamnopyranosyl-(1”’→2”)]-β-D-glucopyranoside (**2**); and 3,4',5’,5-tetrahydroxy-6,7-methylenedioxyflavone-3-O-[α-L-rhamnopyranosyl-(1”’→2”)]-β-D-xylopyranoside (**3**). Compounds **1** and **3** are reported for the first time from nature. The NF-κB luciferase assay showed that these compounds have a partial inhibitory effect on NF-κB activation, compound **2** being the most potent one. In the carrageenan induced paw oedema assay in rats, the flavonoid fraction showed acute anti-inflammatory activity, with the highest percentage of inhibition (75.8%) at a dose of 40 mg/kg.

Keywords: *Boldoa purpurascens*; Nyctaginaceae; flavonoid glycosides; antiinflammatory activity

1. Introduction

Boldoa purpurascens Cav. ex Lag (Nyctaginaceae) is a plant species distributed from northern Mexico and the West Indies to northern South America, Central America and South America. Since it can thrive in disturbed environments it is not considered vulnerable to extinction. In Cuba its leaves are used in a decoction as a diuretic (Roig, 1988) and to treat other kidney disorders. In a previous study we have reported some new flavonoids from the aqueous extract of leaves of *B. purpurascens* (González et al., 2008), as well as anti-inflammatory and antihyperglycemic activities (González et al, 2011; 2013).Herein we describe the isolation of three flavonoid glycosides from the aqueous extract of the leaves of *B. purpurascens* (compounds **1** – **3**), two of which are new, and the evaluation of the anti-inflammatory activity of the flavonoid fraction, one of the isolated constituents (compound **2**), the major flavonoid of *B. purpurascens* isolated before [3,4',5-trihydroxy-6,7-methylenedioxyflavone 3-O-α-L-rhamnopyranosyl-(1'''→2'')-β-D-xylopyranoside] (compound **4**), and the aglycone 3,4',5-trihydroxy-6,7-methylenedioxyflavone obtained after hydrolysis of the major compound.

2. Experimental

2.1. General experimental procedures

1D and 2D NMR spectra were recorded in DMSO-d 6 (compound **3**) and CD_3OD (compounds **1** and **2**) using a Bruker DRX-400 instrument (Rheinstetten, Germany), operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. Chemical shifts are expressed in ppm and coupling constants (*J*) in Hz.

Mass spectra were obtained using an LXQ linear ion trap mass spectrometer (Thermo Fisher, Bremen, Germany) equipped with an ESI source operated in the negative ion mode. Mass spectra of the isolated compounds (**1-3**) were obtained using direct infusion. All data were acquired and processed using Xcalibur software, version 2.0. (Thermo Fisher). Accurate mass measurements were carried out using a QTOF 6530 mass spectrometer (Agilent technologies) equipped with an electrospray ionisation (ESI) source operated in the positive ion mode. Accurate mass measurements were obtained using external calibration. MS spectra of the isolated

compounds (**1-3**) were obtained using direct infusion. All data were acquired and processed using MassHunter software, version B.06.

Specific rotation was determined using a Jasco P-2000 polarimeter (VA Ijsselstein, The Netherlands).

2.2. Plant material

Boldoa purpurascens Cav. ex Lag. was collected in November 2014 at the experimental station ”Las Antillas” of the Las Villas University Cuba and was identified by Cristobal Rios Albuernes, agronomical engineer and specialist of the Botanical Garden of this University. A voucher specimen (No. 3012) is deposited at the Herbarium of the Botanical Garden of this University.

2.3. Isolation of flavonoids

The flavonoid fraction was obtained according to the procedure described by González et al., 2008. The fraction (874 mg) was separated by repeated semi-preparative HPLC with an Autopurification^{TM} system with Quattro Micro-mass detector (Waters) together with a Luna C18 Column (Phenomenex) (250 x 10 mm, 5 µm). The injection volume of a 10 mg/mL 70% methanol solution of the flavonoid mixture was 500 µL and the solvent program was as follows: solvent A: water + 0.1% formic acid; solvent B: acetonitrile + 0.1% formic acid. The gradient conditions were as follows: 0–24 min, 22% B; 25–33 min, 95% B; 34–41 min, 22% B. A flow of 4.75 mL/min was used. Mass spectra were obtained in positive ion mode with following settings: capillary voltage 3.5 KV, cone voltage 50 V, source temperature 140 °C, desolvation temperature 400 °C, desolvation gas flow 800 L/Hr, cone gas flow 50 L/Hr. Compounds were collected based on the *m/z* value of the ions of the aglycone moiety of the flavonoids i.e. *m/z* 317, *m/z* 315, *m/z* 331.

6-Methoxykaempferol-3-O-[α-L-rhamnopyranosyl-(1”’→2”)]-β-D-xylopyranoside ***(1)****:* light yellow solid; $[\alpha]_D^{20}$ -84.57° (DMSO, c 0.36 g/100mL); ^{1}H- and ^{13}CNMR: see Tables 1 and 2; ESI-MS (neg. ion mode): *m/z* 593 $[M - H]^-$; HR-MS (pos. ion mode): *m/z* 617.1430 $[M+Na]^+$ (calculated for $C_{27}H_{30}NaO_{15}$ 617.1477).

*3,4',5-Trihydroxy-6,7-methylenedioxyflavone-3-O-[α-L-rhamnopyranosyl-(1'''→2'')]-β-D-glucopyranoside (**2**):* light yellow solid; $[\alpha]_D^{20}$-110.33° (DMSO, c 0. 66 g/100mL); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2; ESI-MS (neg. ion mode): *m/z* 621 $[M–H]^–$; HR-MS (pos. ion mode): *m/z* 645.1272 $[M+Na]^+$ (calculated for $C_{24}H_{30}NaO_{19}$ 645.1273).

*3,4',5',5-Tetrahydroxy-6,7-methylenedioxyflavone-3-O-[α-L-rhamnopyranosyl-(1'''→2'')]-β-D-xylopyranoside (**3**):* yellow powder; $[\alpha]_D^{20}$-32.52°(DMSO,c 0. 34 g/100mL); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2; ESI-MS (neg. ion mode): *m/z* = 607 $[M–H]^–$; HR-MS (pos. ion mode): *m/z* 631.1252 $[M+Na]^+$ (calculated for $C_{27}H_{27}NaO_{16}$ 631.1270).

2.4. Hydrolysis

The major compound isolated before from *B. purpurascens* [3,4',5-trihydroxy-6,7-methylenedioxyflavone-3-O-α-L-rhamnopyranosyl-(1'''→2'')-β-D- xylopyranoside] (**4)** (González et al., 2008) (18.3 mg) was dissolved in 2 N HCl (H_2O-MeOH 1:1, 18.3 mL) and hydrolysed by heating at 100°C during 3 h. After evaporation of the solvent under vacuum, the residue was suspended in H_2O (36.6 mL) and the mixture extracted with ethyl acetate (3×36.6 mL). The EtOAc phase was evaporated under vacuum to obtain the aglycone3,4',5-trihydroxy-6,7-methylenedioxyflavone (being the same aglycone as compound **2**).

2.5. In vitro NF-kB activity

Cell culture and cell viability (MTT) assay: L929sA fibrosarcoma cells were stably transfected with a NF-κB-driven luciferase reporter gene construct having 3 tandem copies of human NF-κB binding in front of a luciferase reporter gene as previously described (Kaileh et al., 2007) and cultured in DMEM medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37^0C with 5% CO2.In order to determine the effect of these natural compounds on cell viability,L929sA fibrosarcoma cells were subsequently treated with compound **2**, compound **4**, the flavonoid fraction, the aglycone 3,4',5-trihydroxy-6,7-methylenedioxyflavone, quercetin and dexamethasone (positive control) at concentrations of 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 20.0, 40.0 and 80.0 μM for 72 h. The steroidal lactone withaferin

(20μM) was used as reference cytotoxic compound in cell viability assays of aforementioned compounds.

NF-κB luciferase assay: NF-κB dependent luciferase expression in cells treated with various bioactive compounds was measured in different cell lysates by a bioluminescent enzymatic reaction with D-luciferin and ATP (Luciferase Reporter Assay Promega, Cat.# E4550) on an Envision multi-label reader (Perkin Elmer).The relative luciferase activity of each group (three independent experiments) was compared with the TNF stimulated and vehicle control. Dexamethasone was used as a reference anti-inflammatory compound.

2.6. In vivo anti-inflammatory activity

The *in vivo* anti-inflammatory activity of the flavonoid fraction was determined using an acute inflammation experimental model: carrageenan induced paw oedema in rats. Sprague Dawley rats of either sex and of approximately the same age (6–8 weeks), weighing 180–210 g, were used for the anti-inflammatory tests. Animals and commercial pellet diet were provided by The National Center of Production of Laboratory Animals (CENPALB, Cuba). They were housed in polypropylene cages at 25 - 28 ^{o}C with relatively humidity of 45–55%, and were exposed to alternate cycles of 12 h of darkness and light. Food and water were available *ad libitum*. The experimental protocol was approved by the Institutional Ethic Committee of the Experimental Toxicology Unit of Villa Clara Medical School in accordance with institutional guidelines.

Animals of either sex were divided into five groups of 6 animals each. Group I (negative control) received the vehicle only (saline solution 0.9%) and group II (positive control) indomethacin (7 mg/kg, p.o). Animals of groups III, IV and V were treated orally with the flavonoid fraction at doses of 10, 20 and 40 mg/kg, respectively. After 1 h, the rats were challenged with an intradermal injection of 0.1 mL of carrageenan 1% in normal saline solution into the plantar surface of the right hind paw. The paw volume was measured in the beginning of the experiment, and then 2, 3, 4, 5 and 6 h after the carrageenan injection, by means of a plethysmometer (model 7159, Ugo Basile, Italy). The difference between the initial and subsequent reading gave the actual oedema volume. The percentage of inhibition of inflammation was calculated using the formula:

% inhibition = 100 (1 - Vt/Vc), whereVc represents oedema volume in control and Vt oedema volume in the group treated with the test compound.

2.7. Statistical analysis

All valued were expressed as mean ±SD. The data were statistically analyzed using the Kruskal–Wallis, Friedman and Mann–Whitney tests; values $p<0.05$ were considered as statistically significant.

3. Results and discussion

3.1. Structure elucidation

Structures of isolated compounds are shown in Fig. 1. Compound **1** was obtained as a yellow powder. ESI-MS displayed a pseudo-molecular ion $[M-H]^-$ at *m/z* 593. A fragment ion encompassing the aglycone part appeared at *m/z*315. Its structure was established by comparison of the ^{1}H-NMR and ^{13}C-NMR spectra (Tables 1 and 2) with the data of 6-methoxyflavone derivates published earlier (Bertrand et al., 2001) and 2D NMR spectroscopy. The ^{1}H-NMR spectrum in CD_3OD-d_4 (Table 1) showed a typical flavonoid pattern with a *para*-substituted B-ring characterized by two doublets at 8.02 ppm (H-2' and H-6', *J* = 8.97 Hz) and 6.88 ppm (H-3' and H-5', *J*= 8.82 Hz), each integrating for two protons. A substituted A-ring carrying a methoxy group was evident from a singlet at 3.86 ppm, and a singlet at 6.49 ppm indicated the proton for H-8. The remaining signals in the ^{1}H-NMR spectrum indicated the presence of a disaccharide moiety consisting of a pentose attached to the aglycone, and a terminal hexopyranose. In the ^{1}H-NMR spectrum two anomeric protons were observed (δ = 5.61, d, *J* = 7.0 and δ = 5.19, broad singlet); these values were consistent with the β- and α-configuration of the xylose and rhamnose unit, respectively. The position of the glycosidic part could be assigned by the coupling of position 3 (δ_C = 134.0) with the anomeric proton (δ_H = 5.61, d, *J* = 7.0 Hz) of the β-D-xylose in the HMBC spectrum. Both C-1 of the β-D-xylose (δ_C = 101.2) and the α-L-rhamnose (δ_C = 102.7) displayed a coupling with H-2 of β-D-xylose (δ_H = 3.63) in the HMBC spectrum. The COSY spectrum revealed proton-proton correlations between the different protons of the xylose and the rhamnose unit. These correlations together with the doublet at 1.04 ppm (assigned to the methyl group of the rhamnose moiety) confirmed the identity of both sugars. Results from the 2D-NMR spectra as well as comparison with the ^{1}H-

and ^{13}C NMR assignments of related flavonoids observed before in *B. purpurascens* (Gonzalez et al., 2008), confirmed the identity of the discaccharide moiety as α-L-rhamnopyranosyl-(1→2)]-β-D-xylopyranoside. The structure of compound **1** was established as 6-methoxykaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→2)]-β-D-xylo-pyranoside, being a new compound. The analogous compound with a 6,7-methylenedioxy bridge rather than a 6-methoxy, 7-hydroxy substitution pattern had been reported before from *B. purpurascens* (Gonzalez et al., 2008).

Compound **2** was isolated as light yellow powder. The molecular formula was inferred from the ESI-MS, ^{13}C NMR and DEPT data. The ESI-MS spectrum in positive ion mode displayed an $[M–H]^-$ at *m/z* 621, the base peak at *m/z*331represented the aglycone moiety. Close inspection of the ^{1}H- and ^{13}C-NMR data revealed that this compound had been reported before from *B. purpurascens*, although it had been characterized as its acetate (Gonzalez et al., 2008). The aglycone was a 3,4',5-trihydroxy-6,7-methylenedioxyflavone moiety, whereas the disaccharide part was identified as rhamnopyranosyl-(1”’→2”)]-β-D-glucopyranoside residue. The COSY spectrum reveled clear^{1}H-^{1}H correlations between all the protons present in the monosaccharides. More in particular a ^{1}H-^{1}H correlation was observed between H-1” and H-2” (δ 4.02) of the glucose unit. The (1”’→2”) linkage of the rhamosyl to the glucosyl unit was evident from the HMBC correlations observed. H-2” showed a HMBC correlation with the anomeric carbon of the rhamnose moiety at 102.6 ppm. The reverse correlation between C-2” of glucose (δ = 80.04) and H-1”’ of rhamnose (δ = 5.26) was also observed. Therefore, the structure of compound **2** was established as 3,4',5-trihydroxy-6,7-methylenedioxyflavone-3-O-[α-L-rhamno-pyranosyl-(1”’→2”)]-β-D-glucopyranoside.

Compound **3** was obtained as a yellow powder and contained a similar aglycone as compound **2** as far as the A-ring was concerned, but with an additional hydroxy substituent at C-5'. The^{1}H- and ^{13}C-NMR data for the A- and C- rings were consistent with previously isolated compounds from *B. purpurascens*, but some differences could be observed in the B-ring. An additional hydroxylation was observed at position C-5’, confirmed by the chemical shift of C-5’ at 145.24ppm. Two anomeric protons were observed (δ5.52, d, *J* = 7.4 Hz and δ5.08, br s). These values and the chemical shifts of the other protons of the saccharides were consistent with the β- and α-

configuration of a xylose and rhamnose moiety, respectively, as already observed in compound **1** (Tables 1 and 2). The COSY spectrum showed the ^{1}H-^{1}H correlation between H-1 and H-2 (δ 3.57) of the xylose unit, as well as additional correlations between all protons present in the monosaccharides. The disaccharide moiety was identical to the one observed for compound **1**. Due to the agreement of the chemical shifts of all carbons and protons of the glycosidic moiety of compound **3** and compound **1** and data reported of related flavonoids in *B. purpurascens* (Gonzalez et al., 2008)**,** together with 2D-NMR data, the glycosidic moiety could be identified as α-L-rhamnopyranosyl-(1→2)]-β-D-xylopyranoside. As a whole the structure of compound **3** could be established as 3,4',5',5-tetrahydroxy-6,7-methylenedioxy-flavone-3-O-[α-L-rhamnopyranosyl-(1"'→2")]-β-D-xylopyranoside, which is reported for the first time from nature.

3.2. In vitro inhibition of NF-κB luciferase reporter gene expression

The transcription factor NF-κB plays a critical role in normal and pathophysiological immune responses. Therefore, NF-κB and the signaling pathways that regulate its activation have become a major focus of drug development programs. Next, In order to determine the anti-inflammatory activity of the natural compounds, NF-κB-luc reporter cells (Kaileh et al. 2007) were pretreated with the test compounds for 2 h, and then treated with a proinflammatory cytokine TNF-α (10 ng/mL, 2500 IU/ml) or solvent control (DMSO) for 4 h. Compound **2**, Compound **4**, the flavonoid fraction, the aglycone, quercetin, withaferin A and dexamethasone were dissolved in dimethyl sulfoxide (DMSO) and added directly to the culture medium. All test compounds were evaluated at the following concentrations: 1.0, 10.0 and 50.0 μM.

To evaluate the specificity of potential anti-inflammatory effects of the compounds on NF-κB reporter gene assays, cell survival was measured in parallel by the MTT cell survival assay in similar conditions. The results for the MTT and NF-kB luciferase activity are shown in Fig. 2 and 3, respectively.

According to the results, compound **2** weakly inhibited the NF-κB reporter gene induction by TNF, but did not show a dose response behavior. For compound **4**, no significant differences were observed. Furthermore, above a concentration of 10 μM, there is a tendency to decrease the inhibitory effect compared to the dose of 1 μM and no difference was observed between the effect at doses of 1 μM and 50 μM.

The aglycone 3,4',5-trihydroxy-6,7-methylenedioxyflavon was the only test compound that showed inhibition in a dose dependent manner, but according to the results of the MTT assay, at doses of 80μM partial cytotoxicity can be observed. Therefore, it can not be included that inhibition of NF-kB activation observed at doses of 50 μM and higher is partially due to cell death and not to an anti-inflammatory effect of the aglycone 3,4',5-trihydroxy-6,7-methylenedioxyflavone itself.

Many studies report the anti-inflammatory activity of quercetin because of the inhibition of proinflammatory TNF via modulation of the NF-κB system (Ruiz et al., 2007). In this study the effect of quercetin is lower than that of the compound **2**. In general, the compounds have a partial inhibitory effect, whereas compound **2** seems to be the most potent one.

3.3. In vivo anti-inflammatory activity

The anti-inflammatory effect of the flavonoid fraction isolated from *B.purpurascens* in the carrageenan induced rat paw oedema model is shown in Table 3. The Kruskal Wallis test revealed that, generally, there are significant differences between the groups receiving the flavonoid mixture and the control groups ($p < 0.05$).

The carrageenan-induced paw oedema is a useful model in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. Oedema formation in the carrageenan-induced paw oedema model is a biphasic response. In the early hyperemia, 0-2 h after carrageenan injection, there is a release of histamine, serotonin, and bradykinin affecting vascular permeability. The inflammatory oedema reaches its maximum level at the third hour and after that it starts declining (Posadas et al., 2004). Our results revealed that the flavonoid fraction possesses potent activity on the acute phase of inflammation, possibly due to the inhibition of release of inflammatory mediators. Oral administration of the fraction caused a dose-related inhibition of carrageenan induced inflammation.

After two hours no significant difference between the negative control and the lower dose group (10mg/kg) was observed. In all treated groups, except in the positive control, the inflammation increased during 2–3 h, and in general, it started decreasing after 3 h. In the positive control group, the inflammation began to decrease after 2 h. In the experimental group with the highest test dose (40 mg/kg), the decrease was much pronounced after 4 h, and it ended up being smaller than the

positive control. According to that, the flavonoid fraction at dose of 40 mg/kg exhibited a significant anti-inflammatory effect, starting after4 h ($p < 0.05$); the activity was higher than observed for the standard drug indomethacin (7 mg/kg). The experimental groups with lower dose perform better than the negative control but less than the positive control.

When looking at the percentage of inhibition of inflammation in the three experimental groups and the positive control (Fig.4), it was observed that 5 h after initiation of the experiment the percentage of inhibition increased in a dose dependent manner starting from 10 mg/kg. The flavonoid fraction at dose of 40 mg/kg possesses the higher percentage of inhibition (75.8%).

4. Conclusions

Three flavonol glycosides were isolated from the leaves of *Boldoa purpurascens*. These compounds were characterized by spectroscopic analysis as 6-methoxykaempferol-3-*O*-[α-L-rhamnopyranosyl-(1”’→2”)]-β-D-xylopyranoside (**1**); 3,4',5-trihydroxy-6,7-methylenedioxyflavone-3-O-[α-L-rhamnopyranosyl-(1”’→2”)]-β-D-glucopyranoside (**2**); and 3,4',5’,5-tetrahydroxy-6,7-methylenedioxyflavone-3-*O*-[α-L-rhamnopyranosyl-(1”’→2”)]-β-D-xylopyranoside (**3**). Compounds**1** and **3** are reported for the first time from nature. The NF-kB luciferase assay showed that compound **2** possessed a significant anti-inflammatory effect the NF-kB pathway. The flavonoid fraction at 40mg/kg showed the highest acute anti-inflammatory activity in the carrageenan induced rat paw oedema model.

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Table 1. ^{1}H-NMR data for compounds **1**, **2** and **3** (400 MHz)

	Proton	**1 (CD_3OD)**	**2 (CD_3OD)**	**3 (DMSO-d_6)**
Aglycone	Position	δ_H	δ_H	δ_H
	2’	8.02 (d, *J*=8.97 Hz)	8.06 (d, *J*=9.06 Hz)	6.84 (d, *J*=8,81 Hz)
	3’’	6.88 (d, *J*=8.82 Hz)	6.89 (d, *J*=8.91 Hz)	
	5’	6.88 (d, *J*=8.82 Hz)	6.89 (d, *J*=8.91 Hz)	7.56 (t, *)
	6’	8.02 (d, *J*=8.97 Hz)	8.06 (d, *J*=9.06 Hz)	7.57 (t, *)
	4’-OH			
	5-OH			
	8	6.49 (s)	6.67 (s)	6.91 (s)
	CH_3O	3.86 (s)		
	OCH_2O		6.08 (s)	6.17 (s)
β-D-Xylose	Position			
	1	5.61 (d, *J*=7.0 Hz)		5.52 (d, *J*=7.4 Hz)
	2	3.63 (t)		3.57 (t)
	3	3.49 (m)		3.34 (m)
	4	3.48 (m)		3.35 (m)
	5	3.7 (dd, *J*=4.83 Hz, 11.73 Hz) 3.07 (dd, *J*=8.59 Hz, 11.17 Hz)		3.60 (m, *) 2.95 (dd, *J*=8.94 Hz, 11.35 Hz)
α-L- Rhamnose	Position			
	1	5.19 (d, *J*=1.16 Hz)	5.26 (d, *J*=1.23)	5.08 (brs)
	2	3.99 (m)	4.02 (m)	3.73 (m)
	3	3.77 (m)	3.78 (m)	3.48 (m)
	4	3.35 (t)	3.35 (m)	3.14 (m)
	5	4.05 (m)	4.06 (m)	3.78 (m)
	6	1.04 (d, *J*=6.39 Hz)	0.98 (d, *J*=6.25 Hz)	0.87 (d, *J*=6.22 Hz)
β-D-Glucose	Position			
	1		5.75 (d, *J*=7.33)	
	2		3.63 (m)	
	3		3.59 (m)	
	4		3.27 (m)	
	5		3.29 (m)	
	6		3.72 (dd, *J*=2.02 Hz, 11.82 Hz)	

*Could not be determined due to overlap

Table 2. ^{13}C-NMR data for compounds **1**, **2** and **3** (100 MHz)

	Carbon	**1 (CD_3OD)**	**2 (CD_3OD)**	**3 (DMSO-*d_6*)**
Aglycone	Position	δ_C	δ_C	δ_C
	2	158.7	159.0	156.7
	3	134.0	134.4	132.8
	4	179.8	179.8	177.6
	5	153.8	142.5	140.5
	6	132.6	131.0	129.4
	7	158.8	155.7	154.0
	8	94.88	90.2	89.8
	9	153.7	153.7	151.6
	10	106.3	108.8	106.9
	1’	122.9	122.9	120.6
	2’	132.1	132.2	115.3
	3’	116.2	116.1	145.2
	4’	161.5	161.4	149.2
	5’	132.1	132.2	116.1
	6’	116.2	116.1	121.5
	CH_3O	60.9		
	OCH_2O		104.2	102.9
β-$_D$-Xylose	Position			
	1	101.2		99.4
	2	79.6		76.5
	3	78.1		77.0
	4	71.4		69.6
	5	67.1		66.1
α-$_L$- Rhamnose	Position			
	1	102.7	102.6	100.6
	2	72.3	72.4	70.6
	3	72.3	72.2	70.5
	4	74.0	73.9	71.9
	5	70.0	69.9	68.4

	6	17.7	17.5	17.4
β-D-Glucose	Position			
	1		100.2	
	2		80.0	
	3		78.9	
	4		71.8	
	5		78.3	
	6		62.6	

Table 3. Acute anti-inflammatory activity of the test compound and controls in the carrageenan 1% induced rat paw oedema model.

Values are expressed as mean ± SD (n = 6).

	Percentage of inflammation at time (h)				
	1h	2h	3h	4h	5h
NaCl 0.9%	56.14 a	63.26 a	68.79 a	63.26 a	55.00 a
Indomethacin	5.42 e	21.94 d	17.24 d	10.21 d	9.45 c
10 mg/Kg	47.71 b	48.88 a	51.45 b	50.59 b	22.26 a
20 mg/Kg	40.00 c	40.83 b	50.83 b	49.09 b	13.03 b
40 mg/Kg	21.36 d	23.79 c	28.90 c	24.64 c	6.04 d

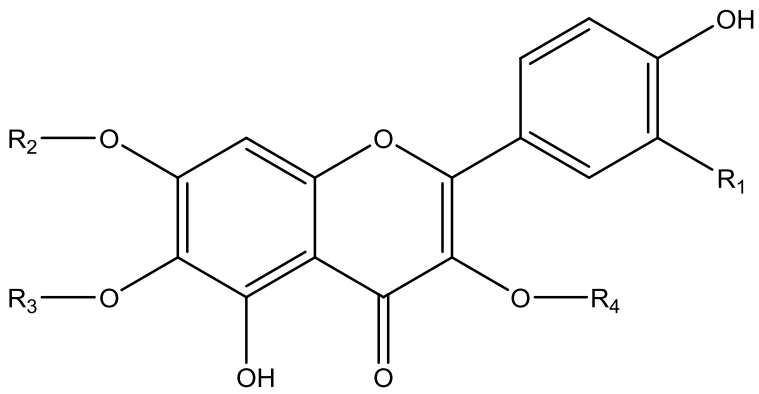

Fig. 1. Structure of compounds **1** – **3**

	R_1	R_2	R_3	R_4
1	H	H	Me	Rha-(1-2)-Xyl
2	H	-CH_2-		Rha-(1-2)-Glc
3	OH	-CH_2-		Rha-(1-2)-Xyl

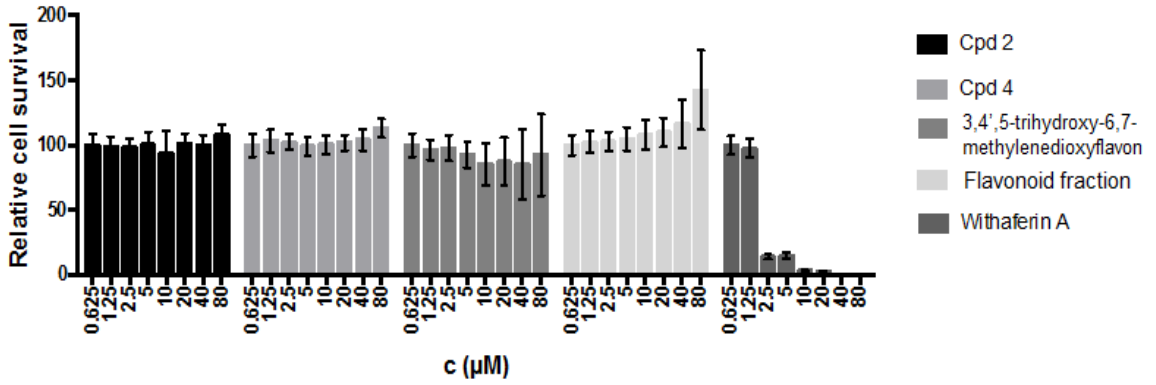

Fig.2. Cell survival MTT assay on L929sA fibrosarcoma cells

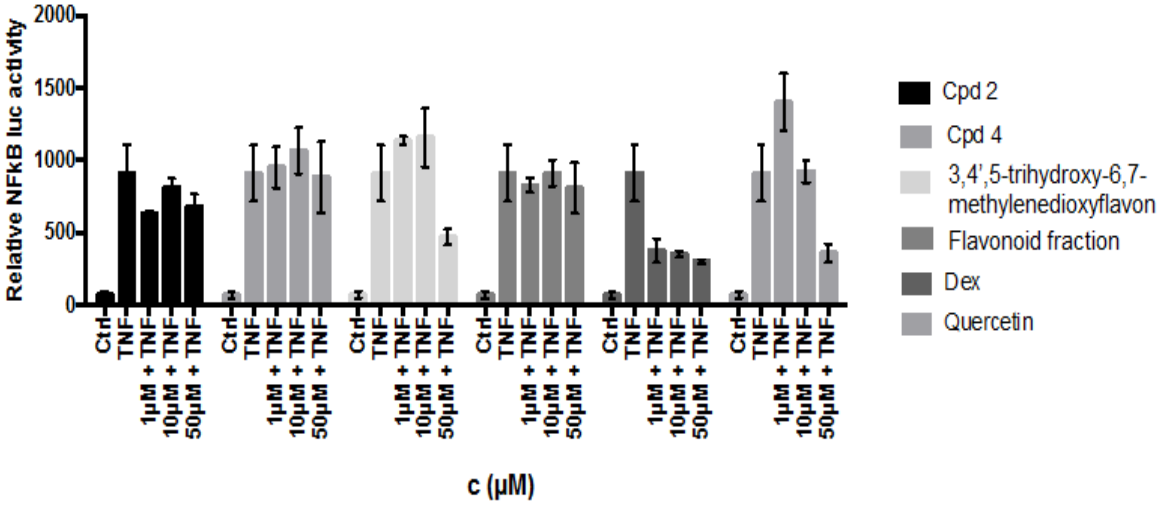

Fig.3. Anti-inflammatory compound effects TNF-induced NF-kB luciferase reporter gene expression in stable transfected L929 fibrosarcoma cells

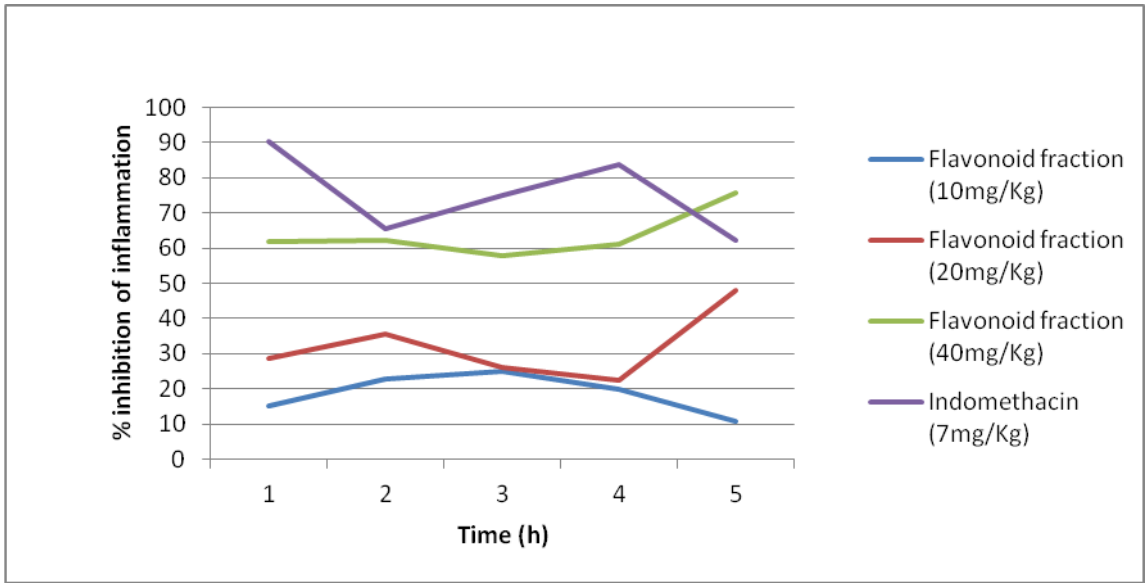

Fig. 4. Percentage of inhibition of inflammation by the flavonoid fraction as a function of time.